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CONTENTS

I. INTRODUCTION

Agropyron is one of the most interesting genera in the tribe of Triticeae due to the following features. Firstly, it is the largest and the most difficult genus of the tribe. It includes about 100 perennial species distributing very widely in the temperate region of the world and comprising many morphologically varied species. Many indigenous species in various parts of the world have been reported. Secondly, Agropyron comprises many species displaying various degrees of ploidy, from 2x to 12x. Of 65 species of Agropyron whose chromosome numbers were examined, tetraploidy was reported in 45 and hexaploidy in 18 species, but diploidy was reported only in ten (Sakamoto 1964b). Thirdly, interspecific and intergeneric hybridization involving Agropyron has been extensively carried out. Knobloch (1963) reported 100 interspecific crosses in Agropyron (including Roegneria) and 172 intergeneric crosses between Agropyron and other genera of the tribe.

Cytogenetical studies of such an interesting genus as Agropyron are of great value for the analysis of variation patterns within the species in connection with their natural habitats. They may also furnish experimental bases for natural biosystematic units and throw a light on speciation processes and evolutionary relationships between the species of the genus.

From these points of view, rather extensive studies on Agropyron have been carried out in Europe (Östergren 1940a, b; Godley 1951; Cauderon 1958; Heneen 1962, 1963), in North America (Stebbins and his coworkers 1946 - 1956; Dewey 1961, 1962, 1963), in South America (Hunziker 1955) and in New Zealand (Conner 1954, 1956, 1957, 1962).

On the contrary, this type of work has not been carried out in Japan, except for some cytogenetical studies of several interspecific hybrids of Agropyron (Matsumura 1941, 1942, 1948).

The present thesis deals with characteristics of the Japanese species, cytogenetical relationships among them in connection with their genomic relationships with Nepalese species and natural hybridization.

Cytological and genetical analyses of hybrids have been of great value in determining the relationships and the origin of many plant species. In Japan five indigenous species of Agropyron are found, three of them tetraploid, Ag. ciliare Franch., Ag. gmelini Scribn. et Smith and Ag. yezoense Honda, and two hexaploid, Ag. humidum Ohwi et Sakamoto and Ag. tsukushiense Ohwi. However, we have no extensive experimental analyses of their cytogenetical relationships or genomic constitution, except Matsumura's cytogenetical observations of hybrids between Ag. tsukushiense var. transiens* and Ag. ciliare or Ag. humidum** (Matsumura 1941, 1942). Therefore, studies along this line have been undertaken by the author.

In the present experiments interspecific hybridization among five indigenous species was carried out in as many combinations as possible. Two tetraploid species collected in Nepal, Ag. gmelini and Ag. semicostatum Nees, which are morphologically very close to the Japanese Ag. gmelini and Ag. tsukushiense (Matsumura, Sakamoto and Tateoka 1956), were used in order to throw a light on the genomic differentiation between Japanese and Nepalese species in relation to their distribution within the same phyto-geographical area, the

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* Matsumura's Ag. semicostatum and ** Ag. Mayebaranum.

Sino-Japanese Region (Good 1953). Moreover, Ag. trachycaulum (Link) Malte, an American tetraploid species belonging to a different phyto-geographical area, was used for a comparison with the Japanese species.

Wild species hybrids provide an important material for taxonomic, genetic and evolutionary studies. Natural hybrids in Agropyron were studied in Europe (Simonet 1934, 1935; Östergren 1940a,b; and others) and in New Zealand (Connor 1956, 1957). In the present study two natural hybrids involving one tetraploid and two hexaploid species of Japanese Agropyron are reported.

II. SPECIES, DISTRIBUTION AND CHROMOSOME NUMBERS OF JAPANESE AGROPYRON

Japanese Agropyron were first described by Miquel (1867) and Franchet and Savatier (1879). They mentioned only two species, one as Triticum caninum Beauv.* and the other as Tr. semicostatum Nees**. Hackel (1899) transferred these two species to Agropyron as Ag. caninum Beauv. and Ag. semicostatum Nees; both have been also described by Makino (1903) and Matsumura (1905).

Honda (1927, 1929, 1930, 1931, 1932, 1935, 1936) studied extensively the classification of the species of this genus occurring in Japan including Taiwan and Korea. In his paper of 1930 he described eight species, Ag. caninum Beauv.*; Ag. ciliare Franch. var. pilosum Honda and var. submuticum Honda; Ag. japonicum Honda var. Hackelianum Honda; Ag. Mayebaranum Honda; Ag. repens Beauv.; Ag. semicostatum Nees**; Ag. yezoense Honda; and Ag. pseudoagropyron Franch. He changed Ag. japonicum into Ag. japonense Honda and added Ag. mite Honda in 1935. Moreover, Honda (1936) described a new species of Agropyron as Elymus tsukushiensis Honda. Later Ohwi (1937a,b, 1941, 1942a,b) reinvestigated this genus, describing eight species, Ag. ciliare Franch. with var. pilosum Honda and var. Hackelianum Honda and forma Okuyamae Ohwi, forma submuticum Ohwi, forma japonense Ohwi and forma mite Ohwi; Ag. Turczaninovii Drob. var. tenuisetum Ohwi; Ag. yezoense var. koryoense (Honda) Ohwi***; Ag. Mayebaranum Honda; Ag. Kamoji Ohwi; Ag. tsukushiense (Honda) Ohwi; Ag. repens

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According to the personal communication from Dr. Ohwi, * refers to a mixture of several species, ** to Ag. semicostatum Miquel, not Nees and *** is incorrect.

Beauv.; and Ag. chinense (Trin.) Ohwi. Finally he made known his conclusive taxonomic treatment of Japanese Agropyron in his "Flora of Japan" published in 1953.

According to Ohwi (1953) and Ohwi and Sakamoto (1964), seven species of Agropyron occur in Japan, among them five indigenous and the other two naturalized, having immigrated from Europe or America. Enumeration and synonyms of the species are given in Table 1. In Table 2 are listed species and variety names, Japanese names, natural habitats, distribution and chromosome numbers of all species.

Ag. repens P. Beauv.: It is distributed in the European mainland and in the Mediterranean region; it was introduced into our country with cereal and forage crops and became naturalized in Hokkaido and Honshu. It is a hexaploid rhizomatous species (2n=42).

Ag. ciliare Franch.: This is a tetraploid species (2n=28), common in Japan. It is also distributed in Korea, China, Manchuria, Siberia and Assam. This species is divided into two varieties, var. minus Ohwi and var. pilosum Honda. The former is found in fields and along road-sides, and the latter occurs mainly in hilly districts. Spikes of the former are nodding and the outer glumes are pubescent. Spikes of the latter are erect with non-pubescent or pubescent only margin of the outer glumes. However, these key characters distinguishing the two varieties are found intermingled in natural populations of the hilly environments. In such places, plants having erect spikes with pubescent glumes or bearing nodding spikes with non-pubescent glumes are found together with typical minus or pilosum forms. The characteristic of this species is that the length of the inner glume is about two thirds of that of the outer glume.

Table 1. Enumeration and synonyms of seven species of Agropyron occurring in Japan

Species	References
1. Agropyron repens (Linn.) P. Beauv.	Honda (1930), Ohwi (1941, 1953)
2. Ag. ciliare (Trin.) Branch.	Honda (1930)
var. minus (Miq.) Ohwi	Ohwi (1953)
= A. ciliare forma Okuyamae Ohwi	Ohwi (1941)
= A. ciliare var. submuticum Honda	Honda (1930, 1931)
= A. ciliare forma submuticum (Honda) Ohwi	Ohwi (1941)
var. pilosum (Korsh.) Honda	Honda (1927, 1930), Ohwi (1941)
= A. japonicum Honda	Honda (1927, 1930)
= A. japonense Honda	Honda (1935)
= A. ciliare forma japonense (Honda) Ohwi	Ohwi (1941)
= A. mite Honda	Honda (1935)
= A. ciliare forma mite (Honda) Ohwi	Ohwi (1941)
= A. japonicum var. Hackelianum Honda	Honda (1927, 1930)
= A. ciliare var. Hackelianum (Honda) Ohwi	Ohwi (1941)
3. Ag. gmelini (Ledeb.) Scribn. et Smith	
var. tenuisetum (Ohwi) Ohwi	Ohwi (1953)
= A. Turczaninovii Drob. var. tenuisetum Ohwi	Ohwi (1941)
4. Ag. caninum P. Beauv.	Ohwi (1953)
5. Ag. yezoense Honda	
var. yezoense	Honda (1929, 1930), Ohwi (1941, 1953)
var. tashiroi (Ohwi) Ohwi	Ohwi (1953)
= A. Tashiroi Ohwi	Ohwi (1937a)
6. Ag. humidum Ohwi et Sakamoto	Ohwi et Sakamoto (1964)
= A. Mayebaranum Honda, pro pte.	Honda (1927, 1930), Ohwi (1941, 1942a, 1953)
7. Ag. tsukushiense (Honda) Ohwi	
var. transiens (Hack.) Ohwi	Ohwi (1953)
= Triticum semicostatum Miquel, non Nees	Miquel (1867), Franch. et Savat. (1879)
= A. semicostatum Hackel, non Nees	Hackel (1899), Makino (1903), Matsumura (1905), Honda (1927, 1930)
= A. Kamoji Ohwi	Ohwi (1942a)
var. tsukushiense	Ohwi (1953)
= Elymus tsukushiensis Honda	Honda (1936)
= A. tsukushiense (Honda) Ohwi	Ohwi (1937b, 1941)
8. Ag. × hatusimae Ohwi	
= A. Hatusimae Ohwi	Ohwi (1942b)
= A. Mayebaranum var. intermedium Hatusima	Ohwi (1953)
9. Ag. × nakasimae Ohwi	
= A. Nakasimae Ohwi	Ohwi (1942b)
= A. Mayebaranum var. Nakasimae (Ohwi) Ohwi	Ohwi (1953)

Table 2. Species, habitat, distribution and chromosome numbers of Agropyron in Japan

Species and variety name	Japanese name	Habitat	Distribution								Chromosome number (2n)	
			Honshu	Shikoku	Kyushu	Hokkaido	Korea	Manchuria	China	Siberia		
Ag. repens P. Beauv.	Shibamugi	fields and road-sides (naturalized)	+			+			+	+	28	Avdulov (1931)
											42	Avdulov (1931)
Ag. ciliare (Trin.) Franch.							+	+	+	+		
var. minus Ohwi	Ao-kamojigusa	fields and road-sides	+	+	+	+					28	Nakajima (1936) Matsumura (1941) Moriya & Kondo (1950) Tateoka (1954)
var. pilosum Honda	Tachi-kamoji	hilly regions	+	+	+						28	Nakajima (1936)
Ag. gmelini (Ledeb.) Scrib. et Smith							+	+	+	+		
var. tenuisetum Ohwi	Inu-kamojigusa	mountain	+						+		28	Ono & Tateoka (1953)
Ag. caninum P. Beauv.	Ibuki-kamojigusa	Mt. Ibuki, Shiga-Ken (naturalized)	+								28	Avdulov (1931) Hartung (1946) Cauderon (1958)
Ag. yezoense Honda												
var. yezoense	Ezo-kamojigusa	fields, hills and mountains	+			+	+	+		+	28	Tateoka (1956)
var. tashiroi (Ohwi) Ohwi	Taishyaku-kamoji	mountains	+				+				-	
Ag. humidum Ohwi et Sakamoto	Mizuta-kamoji	paddy fields and ditches	+		+				+		42	Matsumura (1948) Sakamoto (1958)
Ag. × hatusimae Ohwi	Tarihonoootachi-kamoji	paddy fields and foot path	+		+						42	Sakamoto (1957)
Ag. × nakasimae Ohwi	Zarage-kamoji	dikes, hills	+		+						35	Sakamoto (1958)
Ag. tsukushiense (Honda) Ohwi												
var. transiens (Honda) Ohwi	Kamojigusa	fields and road-sides	+	+	+		+	+	+		42	Nakajima (1936) Matsumura (1941) Moriya & Kondo (1950)
var. tsukushiense	Oni-kamoji	fields			+						-	

Ag. gmelini (Ledeb.) Scrib. et Smith var. tenuisetum Ohwi: It is found only in the mountainous districts of Central Honshu, especially on Mt. Kirigamine, Nagano-Ken. This species (2n=28) seems to be a relic in Japan, however, other varieties of the species are widely distributed in China, Manchuria, Siberia and Nepal. A characteristic distinguishing it from other Japanese species is velvety pubescence and thick rosette leaves in the tillering stage during the winter. This character has also been observed in winter rosette leaves of Nepalese Ag. gmelini and Ag. semicostatum.

Ag. caninum (Linn.) Beauv.: This species is found only on Mt. Ibuki, Shiga-Ken. It was introduced from Europe together with several pharmacological plants in the Tokugawa era. It has become naturalized on this mountain.

Ag. yezoense Honda var. yezoense: It is a tetraploid species (2n=28) and is distributed in Hokkaido, Korea, Manchuria and Siberia, growing in forests, on hills and on river-dikes. It is also found as a relic in deep valleys of the alpine regions of Honshu, such as Kamikochi, Nagano-Ken, Hirogawara, Yamanashi-Ken. A strain collected in Hirogawara had pubescent leaf-sheaths. Another variety, var. tashiroi Ohwi, having no bristles on outer glumes has been found in Taishaku-kyo, Hiroshima-Ken.

Ag. humidum Ohwi et Sakamoto: This species is hexaploid (2n=42) and is restricted to moist paddy fields or ditches in Honshu, Kyushu and China. Adaptation to moist environment is one of the most pronounced characteristics of this species. There have been observed two distinct characters of this species which imply highly adaptiveness to the habitat of fallow paddy fields. One is the formation of

an abscission layer at the first node from the top at maturity. Thus, the upper part from this node on upward is easily removed from the rest of the plant by wind or other physical forces. The other is the perennialization of culm excluding the uppermost internode and spike. New shoots and roots are produced very easily from the nodes when the condition is favorable.

The two characteristics mentioned above have not been found in any of the species belonging to tribe Triticeae of Gramineae which are mostly adapted to rather dry habitats. With these two adaptive traits seeds and clones of this species are dispersed uniformly in the paddy field during the preparation for rice planting in June. This seems to be the reason why this species occurs in swarms and uniform populations in fallow paddy fields as observed in Misima, Shizuoka-Ken (Ohwi and Sakamoto 1964).

Ag. tsukushiense (Honda) Ohwi var. transiens (Hack.) Ohwi: It is the most common hexaploid species (2n=42) in Honshu, Kyushu and Shikoku and is also found in Korea, Manchuria, China, and Loochoc. It grows in fields or along road-sides and shows wide variation in many characters, such as plant habit, anthocyan coloration and waxiness of spike, leaves and culms. Another variety having pubescence on the outer glume, var. tsukushiense, is found in the northern part of Kyushu. A herbarium specimen was kindly sent by Mr. T. Osada, Fukuoka High School, collected in 1955. This plant was fertile and morphologically very similar to var. transiens. Therefore, it seems to be a hairy mutant of Ag. tsukushiense var. transiens.

An ecotype of var. transiens was found as a swarm in the fallow paddy fields in Misima (Sakamoto 1961). The ecotype was distinguished

from the common type in the following characters: pubescent rosette leaves, shorter stature, shorter internodes from top down, shorter flag leaf, spikes and awns; smaller number of spikelets; longer empty glume, lemma and palea; heavier and larger seeds; and very early flowering. Hybrids between the ecotype and the common type were easily obtained, and F_1 and F_2 plants were vigorous and fertile. Genetic differentiation of the ecotype from the common type was observed mainly in the above quantitative characters controlled by a large number of genes or polygenes in addition to several characters governed by single major genes. Since its early flowering was striking, this ecotype was designated as "Early ecotype". The early ecotype was often found together with Chinese milk-vetch (Astragalus sinicus L.) in paddy fields. Its distribution seems to follow that of Chinese milk-vetch.

There have been recognized two putative natural hybrids, Ag. × hatusimae Ohwi and Ag. × nakasimae Ohwi. The former is considered to be a natural hybrid between Ag. humidum and Ag. tsukushiense var. transiens, and the latter is also probably a natural hybrid between Ag. ciliare and Ag. tsukushiense var. transiens. Both are found in Kyushu and Honshu. Detailed observations of these two hybrids confirmed the correctness of these determinations.

A morphological comparison of spikes of the five indigenous species is shown in Table 3 and Fig. 1.

Table 3. Morphological comparison of spikes of five indigenous species (mostly after Ohwi, 1953)

Species / Characters	Ag. ciliare var. minus	Ag. gmelini var. tenuisetum	Ag. yezoense var. yezoense	Ag. humidum	Ag. tsukushiense var. transiens
Shape of spike	rather slender erect or nodding. spikelet appressed	rather slender, erect	rather slender, nodding, spikelets appressed	erect spikelets appressed	broad and bent, spikelets appressed
Length of spike (cm)	middle (10-20)	short (10-15)	short (10-15)	middle (10-20)	long (15-25)
No. of spikelets	14-20	10-15	10-16	10-18	15-26
Length of spikelet (cm)	10-15	15-20	15-20	17-22	15-25
No. of florets per spikelet	4- 7	5- 7	4- 6	4- 7	5-10
Shape of empty glume	Narrow elliptic with 5-7 nerves	wide lanceolate with 5-9 nerves	lanceolate with 3-5 nerves	wide lanceolate with 3-5 nerves	inversely egg-shaped with 3-5 nerves
Length of empty glume (mm)	5- 8	9-11	6- 8	6- 8	5- 8
Pubescence of outer glume	pubescent[1], bristles on margin	non-pubescent	pubescent[2], bristles on margin	non-pubescent	non-pubescent[3]
Length of outer glume (mm)	7-10	9-11	7- 9	9-12	9-12
Awn (mm)	15-20, erect curved when ripe	25-35, erect curved when ripe	15-25, erect rather slender	25-30, erect	20-30, erect
Length of inner glume	2/3-4/5 of outer glume	same as outer glume	same as outer glume	same as outer glume	same as outer glume

1) non-pubescent --- var. pilosum; 2) non-pubescent --- var. tashiroi; 3) pubescent --- var. tsukushiense

Fig. 1. Spikes of five indigenous species of Agropyron in Japan (×0.45)

a. Ag. ciliare var. minus

b. Ag. yezoense var. yezoense

c. Ag. gmelini var. tenuisetum

d. Ag. humidum

e. Ag. tsukushiense var. transiens

III. MATERIALS AND METHODS

Species and strains used in the present study for interspecific crosses are shown in Table 4. Originally seeds of each strain of the Japanese species were collected in various localities except for Ag. ciliare var. minus No. 1 which was collected as a clone. Two tetraploid Nepalese species, Ag. gmelini and Ag. semicostatum, were collected by Dr. S. Nakao, a member of the Japanese Expedition to Nepal-Himalaya in 1952. A tetraploid American Agropyron, Ag. trachycaulum, was sent originally by the Department of Agronomy, College of Washington, Pullman, U. S. A. These materials have been maintained from selfed seeds or as clones in the experimental fields of both the National Institute of Genetics, Misima and Genetics Laboratory, Faculty of Agriculture, Kyoto University, Kyoto.

The hybridization was performed as follows: Usually two lowest florets of the spikelets were emasculated, and the remainder pinched off. The spike was then enclosed in a paraffin-paper bag. After two or three days the emasculated flowers were pollinated by hand.

Cytological studies were made at meiotic divisions of microsporocytes using aceto-carmine squash method. The procedure adopted was as follows:

1. Fixation by acetic alcohol (1:3) for several days or longer.
2. Transfer of the materials into two per cent iron alum solution for 30 minutes followed by washing in water.
3. Staining in aceto-carmine solution for one to several days.
4. Transfer of the material to a slide-glass and after adding a drop of 45 per cent acetic acid applying a cover slip.
5. Gently heating the slide-glass on an alcohol lamp.

Table 4. Species and strains used in interspecific crosses of Agropyron

Species	Strain number	Chromosome number (2n)	Year of collection	Collector	Form	Locality
Ag. ciliare var. minus	No. 1	28	1951	M. Muramatsu	clone	Ashiya, Hyogo-Ken
"	No. 2	28	1952	M. Muramatsu	seeds	Kyoto, Kyoto-Fu
"	No. 3	28	1956	S. Sakamoto	seeds	Misima, Shizuoka-Ken
"	No. 4	28	1956	S. Sakamoto	seeds	Misima, Shizuoka-Ken
"	No. 5	28	1956	S. Sakamoto	seeds	Misima, Shizuoka-Ken
Ag. gmelini var. tenuisetum	-	28	1956	S. Sakamoto	seeds	Kirigamine, Nagano-Ken
Ag. yezoense var. yezoense	-	28	1952	S. Matsumura	seeds	Tokachi-Onsen, Hokkaido
Ag. humidum	No. 1	42	1955	T. Osada	seeds	Fukuoka, Fukuoka-Ken
"	No. 2	42	1956	S. Sakamoto	seeds	Misima, Shizuoka-Ken
Ag. tsukushiense var. transiens	No. 1	42	1937	Y. Nakajima	seeds	Ichinoseki, Iwate-Ken
"	No. 2	42	1956	S. Sakamoto	seeds	Misima, Shizuoka-Ken
Ag. gmelini	-	28	1952	S. Nakao	seeds	Annapurna Base Camp, Nepal
Ag. semicostatum	-	28	1952	S. Nakao	seeds	Tonje, Nepal
Ag. trachycaulum	-	28	1952	Dept. Agronomy, State College of Washington	seeds	Pullman, Washington, U.S.A.

6. Gently tapping the cover slip by the tip of a small pointed wood-peg.

7. Applying pressure with the thumb on the cover slip, keeping it in a fixed position under a strip of filter paper.

8. For obtaining permanent preparations, McClintock's series (McClintock 1929) was used.

Pollen fertility of the hybrids was examined under the microscope after staining by dilute aceto-carmine solution (one volume of aceto-carmine to one volume of 45 per cent acetic acid).

Two natural hybrids, Ag. ciliare × Ag. tsukushiense and Ag. humidum × Ag. tsukushiense, were observed in the suburbs of Misima. Frequency of hybrid occurrence in natural habitats, their natural seed setting and differences in morphology and cytology between artificial and natural hybrids were observed. In Ag. humidum × Ag. tsukushiense, morphology and fertility of the progenies obtained from naturally set seeds were investigated in the experimental field. Seed fertility of natural hybrids was calculated by the following formula, counting seed set in the first, second and third florets in a spikelet. Hence,

$$\text{seed fertility } (\%) = \frac{\text{number of seeds}}{\text{number of spikelets} \times 3} \times 100.$$

IV. EXPERIMENTAL RESULTS AND OBSERVATIONS

(I) Interspecific crosses

Interspecific crosses were carried out in 28 combinations during 1952 - 1957 resulting in 24 hybrids, as shown in Table 5. Ten different crosses, indicated by asterisks in the table, were made by Dr. Mikio Muramatsu in the Genetics Laboratory, Faculty of Agriculture, Kyoto University in 1956.

All cross combinations were classified into seven categories according to the country of occurrence, i.e., Japan, Nepal or North America, and ploidy (tetra- or hexaploid). Thus, all F_1 hybrids were grouped into Japanese 4x × Japanese 4x, Japanese 4x × Nepalese 4x, Nepalese 4x × Nepalese 4x, Japanese 4x × American 4x, Japanese 4x × Japanese 6x, Nepalese 4x × Japanese 6x and Japanese 6x × Japanese 6x.

The difficulty, if any, in obtaining hybrids mainly depended upon differences in the flowering time. For example, the first flowering of Ag. gmelini var. tenuisetum and two Nepalese species, Ag. gmelini and Ag. semicostatum, took place from the end of May to the first ten days of June when high air temperature and humidity do not favor the development of hybrid seeds. Interspecific crosses in the experimental field between them were more difficult than such other crosses.

(II) Morphological and physiological characteristics of the artificial hybrids

The main characteristics of F_1 hybrid plants were the following three.

I. Growth of F_1 plants was vigorous and tillering, heading and flowering were normal. However, only one F_1 plant was obtained

Table 5. Results of interspecific crosses

Combination (♀ × ♂)	Year of cross	No. of flowers crossed	No. of seeds obtained	No. of seeds sown	No. of seeds germinated	Culture no. of F_1 plants
Japanese 4x × Japanese 4x						
Ag. yezoense × Ag. ciliare No. 1	1952	12	4	4	2	R170
Ag. ciliare No. 1 × Ag. yezoense	1955	-	10	10	9	S11
Ag. gmelini × Ag. ciliare No. 3	1957	8	0			-
Japanese 4x × Nepalese 4x						
Ag. ciliare No. 1 × Ag. semicostatum*	1956	24	16	16	16	5731
Ag. semicostatum × Ag. ciliare No. 4	1956	10	1	1	1	5753
Ag. semicostatum × Ag. yezoense*	1956	12	10	9	4	5743
Ag. ciliare No. 4 × Ag. gmelini	1957	20	1	1	1	5878
Ag. gmelini × Ag. ciliare No. 4	1957	14	0			-
Nepalese 4x × Nepalese 4x						
Ag gmelini × Ag. semicostatum	1957	6	2	2	1	5887
Japanese 4x × American 4x						
Ag. ciliare No. 1 × Ag. trachycaulum*	1956	15	11	5	5	5733
Japanese 4x × Japanese 6x						
Ag. tsukushiense No. 1 × Ag. ciliare No. 1*	1956	24	12	11	10	5734
Ag. tsukushiense No. 2 × Ag. ciliare No. 5	1956	64	0			-
Ag. tsukushiense No. 2 × Ag. ciliare No. 3	1958	26	3	3	3	5930
Ag. tsukushiense No. 1 × Ag. yezoense*	1956	22	14	14	12	5736
Ag. yezoense × Ag. tsukushiense No. 1*	1956	24	17	17	6	5742
Ag. gmelini × Ag. tsukushiense No. 2	1957	20	1	1	1	5888
Ag. humidum No. 1 × Ag. ciliare No. 5	1956	100	29	29	19	5752

Combination (♀ ×♂)	Year of cross	No. of flowers crossed	No. of seeds obtained	No. of seeds sown	No. of seeds germinated	Culture no. of F_1 plants
Ag. humidum No. 1 × Ag. ciliare No. 2*	1956	18	0			-
Ag. yezoense × Ag. humidum No. 2	1958	22	0			-
Nepalese 4x × Japanese 6x						
Ag. tsukushiense No. 1 × Ag. semicostatum*	1956	24	19	16	14	5737
Ag. humidum No. 1 × Ag. semicostatum	1957	18	7	7	2	5886
Ag. gmelini × Ag. tsukushiense No. 2	1957	20	12	12	4	5884
Ag. tsukushiense No. 1 × Ag. gmelini*	1956	20	2	2	2	5735
Ag. tsukushiense No. 2 × Ag. gmelini	1957	20	0			-
Ag. gmelini × Ag. humidum No. 1	1957	20	2	2	1	5885
Ag. humidum No. 2 × Ag. gmelini	1957	28	0			-
Japanese 6x × Japanese 6x						
Ag. humidum No. 1 × Ag. tsukushiense No. 1*	1956	14	5	5	5	5741
Ag. humidum No. 1 × Ag. tsukushiense No. 2	1956	76	28	28	17	5751

* Crossed by Dr. Mikio Muramatsu at Faculty of Agriculture, Kyoto University.

between two Nepalese species, Ag. gmelini × Ag. semicostatum (culture no. 5887); it was very weak and grew very slowly. It was difficult to get good spikes for the observation of meiotic divisions of microsporocytes.

2. The general appearance of many characters of F_1 was intermediate between both parents or superior to both, or similar to that of one of the parents, particularly in so far quantitative characters are concerned. For example, 16 quantitative characters of a pentaploid F_1 hybrid between Ag. humidum No. 1 and Ag. ciliare No. 5 (cult. no. 5752) and of a hexaploid F_1 hybrid between Ag. humidum No. 1 and Ag. tsukushiense No. 2 (cult. no. 5751) are given in Table 6 in comparison with those of the parental strains observed in the experimental field in 1957. The results are summarized as follows:

Of 16 characters	Ag. humidum No.1 × Ag. ciliare No.5	Ag. humidum No.1 × Ag. tsukushiense No.2
same as of Ag. humidum No.1	2	1
same as of Ag. ciliare No.5	4	-
same as of Ag. tsukushiense No.2	-	6
intermediate	4	2
superior to both parents	5	6
same as of both parents	1	1
Total	16	16

Out of 16 characters observed, nine of the former, a pentaploid hybrid, and eight of the latter, a hexaploid hybrid, were intermediate or superior to those of both parents indicating hybrid vigor of F_1 hybrids. In these two particular F_1 hybrids, dominance in character expression in F_1 of the female parent of both, i.e. Ag. humidum No. 1,

Table 6. Comparison of quantitative characters of three species and their artificial F_1 hybrids in the experimental fields (1957)

Species / Characters	Parental species			Artificial hybrids	
	Ag. ciliare No. 5	Ag tsukushiense No. 2	Ag. humidum No. 1	Ag. humidum No. 1 × Ag. ciliare No. 5 (culture no. 5752)	Ag. humidum No. 1 × Ag. tsukushiense No. 2 (culture no. 5751)
Plant height (cm)	75.0	90.9	55.9	79.9	91.6
No. of tillers	25.0	42.6	103.6	106.0	61.6
Length of the first internode from top (cm)	44.2	53.3	30.3	48.4	56.5
Flag leaf Length (cm)	16.9	17.4	9.2	15.1	16.2
Flag leaf Width (cm)	1.1	1.2	0.5	1.0	1.0
Length of spike (cm)	20.9	26.3	16.8	24.5	30.6
No. of spikelets	19.9	23.6	12.6	18.2	20.6
No. of florets per spikelet	10.3	7.6	6.0	6.9	7.5
Date of first heading	May 29	May 21	May 17	May 21	May 20
Date of flowering	May 22	May 21	May 16	May 20	May 19
Empty glume Length (cm)	0.66	0.91	0.89	0.97	0.87
Empty glume Width (cm)	0.17	0.13	0.14	0.16	0.15
Outer glume with awn Length (cm)	2.47	3.92	3.32	3.83	4.21
Outer glume with awn Width (cm)	0.17	0.18	0.16	0.17	0.18
Inner glume Length	0.49	0.92	0.92	0.88	0.99
Inner glume Width	0.14	0.12	0.12	0.15	0.16
Seed Length (cm)	0.45	0.54	0.53		
Seed Width (cm)	0.15	0.16	0.14	(sterile)	(sterile)
Seed Weight (mg)	2.6	4.8	5.5		

was found only in two characters of the pentaploid and in one character of the hexaploid hybrid. On the contrary, dominance of the male parent appeared in four characters of the former and in six of the latter. Spikes of F_1 hybrids and their parental species are shown in Figs. 2, 3, 4 and 5.

In two reciprocal interspecific crosses, Ag. ciliare No. 1 × Ag. yezoense (cult. nos. R170 and S11) and Ag. tsukushiense No. 1 × Ag. yezoense (cult. nos. 5736 and 5742), no significant morphological differences between reciprocal crosses were observed (Fig. 2a,b and Fig. 3b,c).

3. Complete pollen sterility and high ovule sterility were the rule in all combinations of interspecific hybrids regardless of the chromosome number of the parents. All anthers were nondehiscent at the flowering time and all pollen grains were completely abortive and without contents. No seeds were obtained from self-pollination. However, 14 seeds were obtained from open-pollinated flowers of only one F_1 hybrid between Ag. humidum No. 1 and Ag. tsukushiense No. 2. Total 1,102 spikelets of 60 spikes were examined. Taking into consideration the first, second and third florets in a spikelet for the calculation of seed fertility, 0.42 per cent seed fertility was obtained from open-pollination. Backcrosses were tried in nine interspecific combinations and two seeds were obtained only when Ag. humidum No. 1 was backcrossed to F_1 between Ag humidum No. 1 and Ag. tsukushiense No. 2.

(III) Chromosome pairing in the artificial hybrids

1. Tetraploid hybrids

(1) Japanese 4x × Japanese 4x: Ag. yezoense × Ag. ciliare No. 1 (cult. no. R170) was examined cytologically. The range of bivalent

Fig. 2. Spikes of F_1 hybrids and parental species: Japanese 4x × Japanese 4x, Japanese 4x × Nepalese 4x, Nepalese 4x × Nepalese 4x and Japanese 4x × American 4x. (×0.4)

a. Ag. ciliare No. 1 × Ag. yezoense (R170)
b. Ag. yezoense × Ag. ciliare No. 1 (S11)
c. Ag. ciliare No. 1 × Ag. semicostatum (5731)
d. Ag. semicostatum × Ag. yezoense (5743)
e. Ag. ciliare No. 4 × Ag. gmelini (Nepal) (5878)
f. Ag. gmelini (Nepal) × Ag. semicostatum (5887)
g. Ag. ciliare No. 1 × Ag. trachycaulum (5733)
h. Ag. ciliare
i. Ag. yezoense
j. Ag. gmelini (Nepal)
k. Ag. semicostatum
l. Ag. trachycaulum

Fig. 3. Spikes of F_1 hybrids and parental species: Japanese 4x × Japanese 6x. (×0.4)

a. Ag. tsukushiense No. 1 × Ag. ciliare No. 1 (5734)
b. Ag. tsukushiense No. 1 × Ag. yezoense (5736)
c. Ag. yezoense × Ag. tsukushiense No. 1 (5742)
d. Ag. gmelini (Japan) × Ag. tsukushiense No. 2 (5888)
e. Ag. humidum No. 1 × Ag. ciliare No. 5 (5752)
f. Ag. ciliare
g. Ag. gmelini (Japan)
h. Ag. yezoense
i. Ag. humidum
j. Ag. tsukushiense

Fig. 4. Spikes of F_1 hybrids and parental species:
Nepalese 4x × Japanese 6x. (×0.4)

a. Ag. tsukushiense No. 1 × Ag. semicostatum (5737)
b. Ag. humidum No. 1 × Ag. semicostatum (5886)
c. Ag. gmelini (Nepal) × Ag. tsukushiense No. 2 (5884)
d. Ag. tsukushiense No. 1 × Ag. gmelini (Nepal) (5735)
e. Ag. gmelini (Nepal) × Ag. humidum No. 1 (5885)
f. Ag. gmelini (Nepal)
g. Ag. semicostatum
h. Ag. humidum
i. Ag. tsukushiense

Fig. 5. Spikes of F_1 hybrid and parental species:
Japanese 6x × Japanese 6x. (×0.45)

a. Ag. humidum No. 1

b. Ag. humidum No. 1 × Ag. tsukushiense No. 2 (5751)

c. natural hybrid in Misima (H_1)

d. Ag. tsukushiense No. 2

pairing was 10 - 14_{II} with the mode at 14 (Tables 7 and 20). In 45 plates examined, one quadrivalent was observed in 14 cells, in one cell two quadrivalents were found, one trivalent was detected in seven cells and two trivalents were observed in two cells. Hence, relatively high quadrivalent association was found in this combination.

(2) Japanese 4x × Nepalese 4x: In this case, three hybrid combinations, Ag. ciliare No. 1 × Ag. semicostatum (cult. no. 5731), Ag. semicostatum × Ag. yezoense (cult. no. 5743) and Ag. ciliare No. 4 × Ag. gmelini (Nepal) (cult. no. 5878) were studied. The results for the first two combinations are given in Tables 7 and 20. Variation of bivalent formation in the first hybrid was from 5_{II} to 14_{II}. More quadrivalents and trivalents were found in the first hybrid than in the second one. In the third hybrid, Ag. ciliare No. 4 × Ag. gmelini (Nepal), only 16 MI plates could be observed and $1_{III} + 11_{II} + 3_{I}$, $12_{II} + 4_{I}$, $1_{III} + 12_{II} + 1_{I}$, $1_{IV} + 11_{II} + 2_{I}$, $13_{II} + 2_{I}$, $1_{IV} + 1_{III} + 10_{II} + 1_{I}$ and 14_{II} were obtained in the frequency of 1, 1, 1, 1, 3, 1, and 8 respectively.

(3) Nepalese 4x × Nepalese 4x: Only one F_1 plant of the hybrid, Ag. gmelini × Ag. semicostatum (cult. no. 5887) was obtained but it was very weak. No cytological observation was made.

(4) Japanese 4x × American 4x: An interspecific hybrid (cult. no. 5733) between Ag. ciliare No. 1 × Ag. trachycaulum was obtained. Chromosome pairing in F_1 is given in Table 8. The result shows a very small number of bivalent association ranging from 2_{II} to 9_{II} with 5 as the mode (Fig. 6e,f). Average number of univalents was 17 per meiotic plate and a few quadrivalents and trivalents were observed (Table 20).

Table 7. Chromosome pairing in F_1 hybrids among Ag. ciliare, Ag. yezoense and Ag. semicostatum

Cross combination	Ag. ciliare No. 1 × Ag. semicostatum (5731)		Ag. semicostatum × Ag. yezoense (5743)		Ag. ciliare No. 1 × Ag. yezoense (R170)	
Chromosome pairing	Frequency		Frequency		Frequency	
$5_{II}+18_{I}$	1	1 (0.2%)				
$6_{II}+16_{I}$	2	2 (0.2)				
$7_{II}+14_{I}$	2					
$1_{III}+6_{II}+13_{I}$	3	5 (0.5)	1	1 (1.5%)		
$8_{II}+12_{I}$	4					
$1_{III}+7_{II}+11_{I}$	2					
$1_{IV}+6_{II}+12_{I}$	1	8 (1.2)				
$1_{IV}+1_{III}+5_{II}+11_{I}$	1					
$9_{II}+10_{I}$	6					
$1_{III}+8_{II}+9_{I}$	4					
$1_{IV}+7_{II}+10_{I}$	1	12 (2.9)				
$1_{V}+7_{II}+9_{I}$	1					
$10_{II}+8_{I}$	23		2	2 (3.0)	1	1 (2.2%)
$1_{III}+9_{II}+7_{I}$	8	33 (8.1)				
$1_{IV}+8_{II}+8_{I}$	2					
$11_{II}+6_{I}$	60		6		2	
$1_{III}+10_{II}+5_{I}$	17	86 (21.1)	1	7 (10.1)		
$1_{IV}+9_{II}+6_{I}$	9				1	5 (11.1)
$1_{IV}+1_{III}+8_{II}+5_{I}$					2	
$12_{II}+4_{I}$	94		27		6	
$1_{III}+11_{II}+3_{I}$	18		1		1	
$2_{III}+10_{II}+2_{I}$		130 (31.9)		29 (42.0)	1	12 (26.7)
$1_{IV}+10_{II}+4_{I}$	18				4	
$1_{IV}+1_{III}+9_{II}+3_{I}$			1			
$13_{II}+2_{I}$	73		17	17 (24.6)	7	
$1_{III}+12_{II}+1_{I}$	8	96 (23.5)			2	
$1_{IV}+11_{II}+2_{I}$	15				2	13 (28.9)
$1_{IV}+1_{III}+10_{II}+1_{I}$					2	
14_{II}	27		11		10	
$1_{IV}+12_{II}$	8	35 (8.6)	2	13 (18.8)	3	14 (31.1)
$2_{IV}+10_{II}$					1	
Total	408		69		45	

Table 8. Chromosome pairing in the F_1 hybrid between *Ag. ciliare* No. 1 and *Ag. trachycaulum* (5733)

Chromosome pairing	Frequency		
$2_{II} + 24_I$	3	3	(0.9%)
$3_{II} + 22_I$	15	15	(4.6)
$4_{II} + 20_I$	54		
$1_{III} + 3_{II} + 19_I$	1	55	(16.9)
$5_{II} + 18_I$	96		
$1_{III} + 4_{II} + 17_I$	5	101	(31.1)
$6_{II} + 16_I$	78		
$1_{III} + 5_{II} + 15_I$	6		
$2_{III} + 4_{II} + 14_I$	1	86	(26.5)
$1_{IV} + 4_{II} + 16_I$	1		
$7_{II} + 14_I$	43		
$1_{III} + 6_{II} + 13_I$	5	51	(15.7)
$1_{IV} + 5_{II} + 14_I$	3		
$8_{II} + 12_I$	11		
$1_{III} + 7_{II} + 11_I$	1	13	(4.0)
$1_{IV} + 6_{II} + 12_I$	1		
$9_{II} + 10_I$	1	1	(0.3)
Total	325		

Fig. 6. Chromosome pairing in F_1 hybrids, Ag. ciliare No. 1 × Ag. yezoense, Ag. ciliare No. 1 × Ag. semicostatum and Ag. ciliare No. 1 × Ag. trachycaulum. (×1,600)

a. Ag. yezoense × Ag. ciliare No. 1: $1_{IV} + 11_{II} + 2_I$
b. Ag. ciliare No. 1 × Ag. semicostatum: $10_{II} + 8_I$
c. " : $1_{IV} + 10_{II} + 4_I$
d. " : 14_{II}
e. Ag. ciliare No. 1 × Ag. trachycaulum: $5_{II} + 18_I$
f. " : $5_{II} + 18_I$

2. Pentaploid hybrids

(1) Japanese 4x × Japanese 6x: Interspecific hybrids were obtained in six hybrid combinations in this group. Cytological examination of the F_1 hybrids was made in five combinations including a reciprocal crosses between Ag. tsukushiense No. 1 and Ag. yezoense, as shown in Tables 9 and 20. Range of bivalent fluctuation was 7_{II}-16_{II} and the mode of bivalent association was 13_{II} or 14_{II} in these Japanese pentaploid hybrids (Fig. 7).

Chromosome pairing in the reciprocal crosses between Ag. yezoense and Ag. tsukushiense, cult. nos. 5736 and 5742, was very similar as shown in Table 9, suggesting that no significant reciprocal differences occurred.

(2) Nepalese 4x × Japanese 6x: Five interspecific hybrids were obtained in 1956 and 1957 (Table 5). Chromosome pairing of four kinds of F_1 hybrids in this group was observed as shown in Table 10. The results of cytological observations in this group were distinctly different from those found in Japanese pentaploid hybrids concerning the following points;

1) wider range of bivalent fluctuation from 3_{II} to 15_{II},
2) lower average number of bivalents, 10_{II} - 12_{II}, and
3) higher number of univalent formation, 11_I - 14_I, as shown in Table 20. Chromosome pairing in F_1 hybrid of Ag. tsukushiense No. 1 × Ag. semicostatum is shown in Fig. 8.

No significant difference in the frequency of multivalent association was detected between Japanese 4x × Japanese 6x and Nepalese 4x × Japanese 6x (Table 20)

3. Hexaploid hybrid

Table 9. Chromosome pairing in F_1 hybrids among Ag. ciliare, Ag. yezoense, Ag. gmelini (Japan), Ag. tsukushiense and Ag. humidum

Cross combination / Chromosome pairing	Ag. tsukushiense No. 1 × Ag. ciliare No. 1 (5734)	Ag. tsukushiense No. 1 × Ag. yezoense (5736)	Ag. yezoense × Ag. tsukushiense No. 1 (5742)	Ag. gmelini (Japan) × Ag. tsukushiense No. 2 (5888)	Ag. humidum No. 1 × Ag. ciliare No. 5 (5752)
	Frequency	Frequency	Frequency	Frequency	Frequency
$1_{III}+6_{II}+18_{I}$		1 1 (1.2%)			
$9_{II}+17_{I}$	1 1 (0.8%)				
$10_{II}+15_{I}$	2 2 (1.6)				
$1_{IV}+1_{III}+7_{II}+14_{I}$		1 1 (1.2)			
$11_{II}+13_{I}$	4, 1 → 5 (4 0)	1, 4, 1, 1, 2 → 9 (10.3)	1, 1, 1 → 3 (5.5%)	1, 1 → 2 (4.0%)	
$1_{III}+10_{II}+12_{I}$					
$1_{IV}+9_{II}+13_{I}$					
$2_{III}+9_{II}+11_{I}$					
$1_{IV}+1_{III}+8_{II}+12_{I}$					4 4 (7.5%)
$12_{II}+11_{I}$	8, 2 → 10 (8.0)	8, 7, 2, 1 → 18 (20.7)	2, 2, 2, 1 → 7 (12.7)	9, 2, 2, 1 → 14 (28.0)	
$1_{III}+11_{II}+10_{I}$					
$2_{III}+10_{II}+9_{I}$					
$1_{IV}+10_{II}+11_{I}$					
$1_{IV}+1_{III}+9_{II}+10_{I}$					
$13_{II}+9_{I}$	30, 2, 3 → 35 (28.0)	16, 5, 4 → 25 (28.7)	9, 6, 1, 1 → 17 (30.9)	13, 3, 2 → 18 (36.0)	8, 1 → 9 (17.0)
$1_{III}+12_{II}+8_{I}$					
$1_{IV}+11_{II}+9_{I}$					
$1_{V}+11_{II}+8_{I}$					

Cross combination / Chromosome pairing	Ag.tsukushiense No.1 × Ag.ciliare No.1 (5734)	Ag.tsukushiense No.1 × Ag.yezoense (5736)	Ag.yezoense × Ag.tsukushiense No.1 (5742)	Ag.gmelini (Japan) × Ag.tsukushiense No.2 (5888)	Ag.humidum No.1 × Ag.ciliare No.5 (5752)
	Frequency	Frequency	Frequency	Frequency	Frequency
$14_{II}+7_{I}$	46	24	18	10	28
$1_{III}+13_{II}+6_{I}$	3 → 52 (41.6)	2	1 → 25 (45.4)	3 → 14 (28.0)	3 → 31 (56.4)
$1_{IV}+12_{II}+7_{I}$	3	1 → 28 (32.2)	6	1	
$2_{IV}+10_{II}+7_{I}$		1			
$1_{IV}+1_{III}+10_{II}+6_{I}$					
$15_{II}+5_{I}$	2 2 (1.6)	2		2 2 (4.0)	6
$1_{IV}+13_{II}+5_{I}$		3 → 5 (5.7)	3 3 (5.5)		2 → 8 (15.1)
$16_{II}+3_{I}$					1 1 (1.9)
Total	107	87	55	50	53

Table 10. Chromosome pairing in F_1 hybrids among Ag. semicostatum, Ag. gmelini (Nepal), Ag. tsukushiense and Ag. humidum

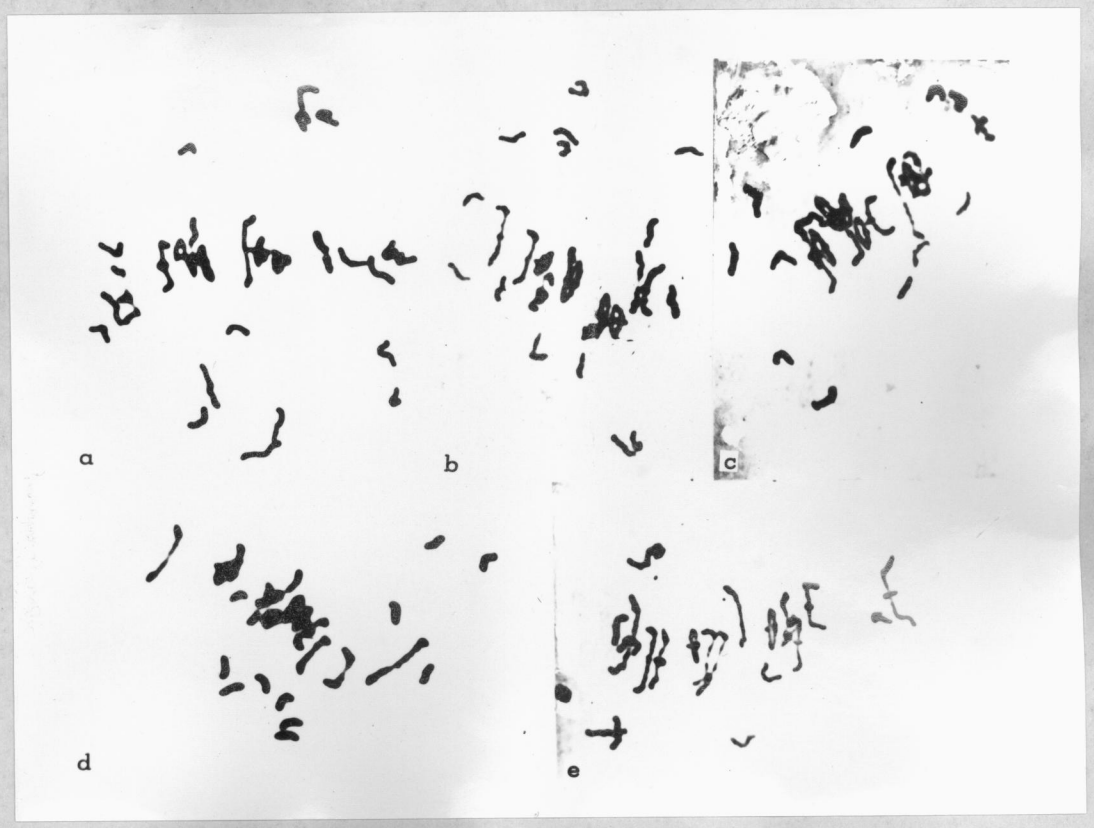
Cross combination / Chromosome pairing	Ag. tsukushiense No 1 × Ag. semicostatum (5737) Frequency	Ag. humidum No. 1 × Ag. semicostatum (5886) Frequency	Ag. gmelini (Nepal) × Ag. tsukushiense No. 2 (5884) Frequency	Ag. gmelini (Nepal) × Ag. humidum No. 1 (5885) Frequency
$3_{II}+29_I$			1 1 (1.0%)	
$4_{II}+27_I$				
$5_{II}+25_I$			1 1 (1.0)	
$6_{II}+23_I$	1		1 1 (1.0)	
$1_{III}+5_{II}+22_I$	1 3 (1.9%)			
$1_{IV}+4_{II}+23_I$	1			
$7_{II}+21_I$	5		2 2 (2.1)	
$1_{III}+6_{II}+20_I$	1 7 (4.5)			
$1_{IV}+1_{III}+4_{II}+20_I$	1			
$8_{II}+19_I$	13	1 1 (2.1%)	2 2 (2.1)	
$1_{III}+7_{II}+18_I$	2 15 (9.7)			
$9_{II}+17_I$	23	10 10 (21.3)	5	1 1 (4.0%)
$1_{III}+8_{II}+16_I$	3 28 (18.0)		1 6 (6.2)	
$1_{IV}+7_{II}+17_I$	2			
$10_{II}+15_I$	26	15 15 (31.9)	8	1 1 (4.0)
$1_{III}+9_{II}+14_I$	2 30 (19.4)		3 13 (13.4)	
$1_{IV}+8_{II}+15_I$	2		2	

Cross combination	Ag. tsukushiense No. 1 × Ag. semicostatum (5737)	Ag. humidum No. 1 × Ag. semicostatum (5886)	Ag. gmelini (Nepal) × Ag. tsukushiense No. 2 (5884)	Ag. gmelini (Nepal) × Ag. humidum No. 1 (5885)
Chromosome pairing	Frequency	Frequency	Frequency	Frequency
$11_{II}+13_{I}$	22	8 8 (17.0)	14	7
$1_{III}+10_{II}+12_{I}$	8		1 16 (16.5)	2 9 (36.0)
1_{IV} + $9_{II}+13_{I}$	6 37 (23.9)		1	
$1_{IV}+1_{III}+8_{II}+12_{I}$	1			
$12_{II}+11_{I}$	16	6	15	3 3 (12.0)
$1_{III}+11_{II}+10_{I}$	5 25 (16.1)	1 7 (14.9)	1 18 (18.6)	
1_{IV} $+10_{II}+11_{I}$	4		2	
$13_{II}+9_{I}$	7	2 2 (4.3)	10	6
$1_{III}+12_{II}+8_{I}$	1 8 (5.2)		2	
$2_{III}+11_{II}+7_{I}$			1 14 (14.4)	7 (28.0)
1_{IV} $+11_{II}+9_{I}$			1	1
$14_{II}+7_{I}$	2 2 (1.3)	4 4 (8.5)	12	3 3 (12.0)
$1_{III}+13_{II}+6_{I}$			1 15 (15.5)	
1_{IV} $+12_{II}+7_{I}$			2	
$15_{II}+5_{I}$			5	1 1 (4.0)
1_{IV} $+13_{II}+5_{I}$			3 8 (8.2)	
Total	155	47	97	25

Fig. 7. Chromosome pairing in F_1 hybrids, Ag. tsukushiense No. 1 × Ag. ciliare No. 1, Ag. tsukushiense No. 1 × Ag. yezoense, Ag. gmelini (Japan) × Ag. tsukushiense No. 2 and Ag. humidum No. 1 × Ag. ciliare No. 5. (×1,600)

a. Ag. tsukushiense No. 1 × Ag. ciliare No. 1: $14_{II} + 7_I$
b. Ag. tsukushiense No. 1 × Ag. yezoense: $14_{II} + 7_I$
c. Ag. humidum No. 1 × Ag. ciliare No. 5: $14_{II} + 7_I$
d. Ag. gmelini (Japan) × Ag. tsukushiense No. 2: $1_{IV} + 11_{II} + 9_I$

Fig. 8. Chromosome pairing in F_1 hybrid, *Ag. tsukushiense* No. 1 × *Ag. semicostatum*. (×1,600)



a. $1_{III} + 7_{II} + 18_{I}$
b. $10_{II} + 15_{I}$
c. $11_{II} + 13_{I}$
d. $12_{II} + 11_{I}$
e. $12_{II} + 11_{I}$

Japanese 6x × Japanese 6x: Since two hexaploid species are distributed in Japan, only one interspecific hybrid, Ag. humidum × Ag. tsukushiense, was investigated. The result of cytological observation of the F_1 hybrids between Ag. humidum No. 1 × Ag. tsukushiense No. 2 (cult. no. 5751) is given in Table 11. Most of meiotic figures of the hybrid showed 21_{II} (Fig. 9a) as observed in the parents, and the range of bivalents was 19_{II} - 21_{II} with very low frequency of multivalents and univalents.

Table 11. Chromosome pairing in the F_1 hybrid between Ag. humidum No. 1 and Ag. tsukushiense No. 2

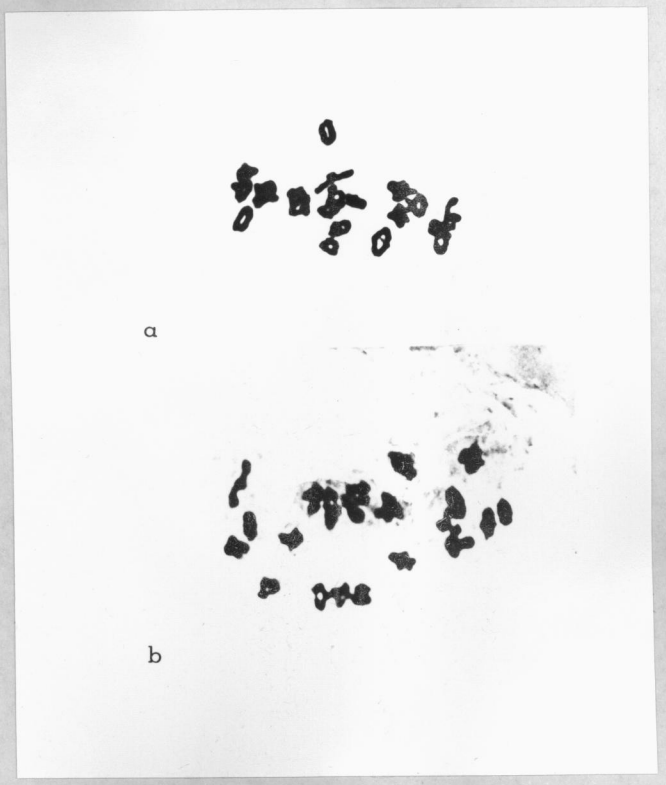
Chromosome pairing	21_{II}	$20_{II} + 2_I$	$1_{III} + 19_{II} + 1_I$	Total
Frequency	35	10	1	46

(IV) Natural hybrids between Ag. ciliare and Ag. tsukushiense

Ag. tsukushiense var. transiens and Ag. ciliare are common species in Japan, distributed in Honshu, Kyushu and Shikoku. The former is a hexaploid growing in fields and along road-sides, and the latter is a tetraploid found mainly in fields and in hilly districts.

In the sympatric places of the two species, mainly in the hills and on river dikes in the suburbs of Misima, many clones of natural hybrids were found as shown in Fig. 10. As many as 32 clones were found on the river dikes of the Sakai River within about 1,000 meters. Most of the natural hybrid clones were very vigorous, probably due to hybrid vigor. Seed setting of 23 clones of natural hybrids was observed in 1959 and Table 12 lists seed fertility of each clone.

Fig. 9. Chromosome pairing in an artificial F_1 hybrid and a natural hybrid of *Ag. humidum* × *Ag. tsukushiense*. (×1,600)



a. *Ag. humidum* No. 1 × *Ag. tsukushiense* No. 2: 21_{II}

b. A natural hybrid (a-7) of *Ag. humidum* × *Ag. tsukushiense* collected in Fukuoka: 21_{II}

Fig. 10. Distribution of the natural hybrids between Ag. ciliare and Ag. tsukushiense var. transiens in the suburb of Misima.

Black circles indicate clones of the natural hybrid. White parts are paddy fields. Dotted parts are upland fields or woods in the hilly regions. Hatched parts indicate towns or villages.

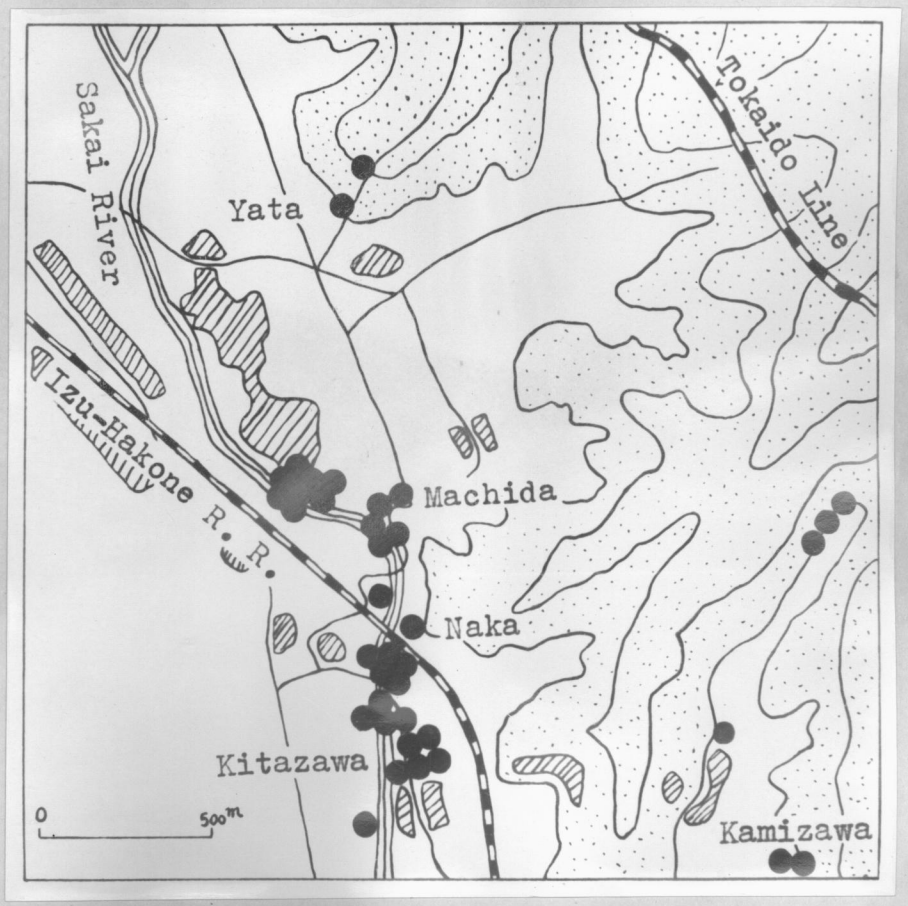


Table 12. Seed fertility of natural hybrids between Ag. tsukushiense var. transiens and Ag. ciliare at natural habitats in Misima.

Strain	Locality	Year observed	No. of spikes	No. of spikelets	No. of seed	Seed fertility (%)
H_{25-1}	Kitazawa	1959	12	251	0	0
-2	"	"	16	413	0	0
H_{27-1}	"	"	34	601	0	0
-2	"	"	5	91	0	0
-3	"	"	24	481	0	0
H_{28-1}	"	"	2	28	0	0
-2	"	"	9	213	0	0
-3	"	"	3	71	0	0
H_{34}	Kamizawa	"	48	798	1*	-
H_{37}	Machida	"	1	21	0	0
H_{38-1}	"	"	14	182	0	0
-2	"	"	1	20	0	0
H_{39}	"	"	4	94	0	0
H_{40}	Kitazawa	"	19	442	0	0
H_{41-1}	Machida	"	24	462	0	0
-2	"	"	17	323	0	0
-3	"	"	6	106	0	0
-4	"	"	4	69	0	0
-5	"	"	5	76	0	0
-6	"	"	15	317	0	0
-7	"	"	7	119	0	0
-8	"	"	9	122	0	0
H_{42}	Naka	"	2	27	0	0

* A very small underdeveloped seed.

All clones observed were completely sterile and no seed was obtained except only one, from H_{34}. This seed was very small, soft and underdeveloped.

The natural and the artificial hybrids were morphologically similar and somewhat resembled the Ag. tsukushiense parent, but their outer glume was pubescent which is a characteristic trait of Ag. ciliare.

Chromosome pairing in meiosis of a natural hybrid plant (H_{13}) was examined as shown in Table 13; the same configurations of chromosomes as observed in the artificial hybrid were found (cf. Table 9). On the average the chromosome pairing at MI was $14_{II} + 7_{I}$.

(V) Natural hybrids between Ag. humidum and Ag. tsukushiense

Two Japanese Agropyron species, Ag. humidum and Ag. tsukushiense var. transiens, are both hexaploid. Ag. humidum is restricted to moist paddy fields or ditches, while Ag. tsukushiense is found along road-sides and in fields. Thus, the two species are considered as allopatric, occurring in nature in different ecological habitats. However, an early ecotype of Ag. tsukushiense which differs from the common type of this species has been found (Sakamoto 1961). It has a sympatric relationship to Ag. humidum in moist paddy fields. For this reason allopatric and sympatric cases of natural hybridization between Ag. humidum and Ag. tsukushiense were expected. Field observations have confirmed the occurrence of both in the vicinity of Misima.

a) Allopatric case --- Natural hybrids between Ag. humidum and the common type of Ag. tsukushiense

During the mapping of the distribution of Ag. humidum in the

Table 13. Chromosome pairing at MI of a natural hybrid (H_{13}) between Ag. tsukushiense and Ag. ciliare collected in Misima

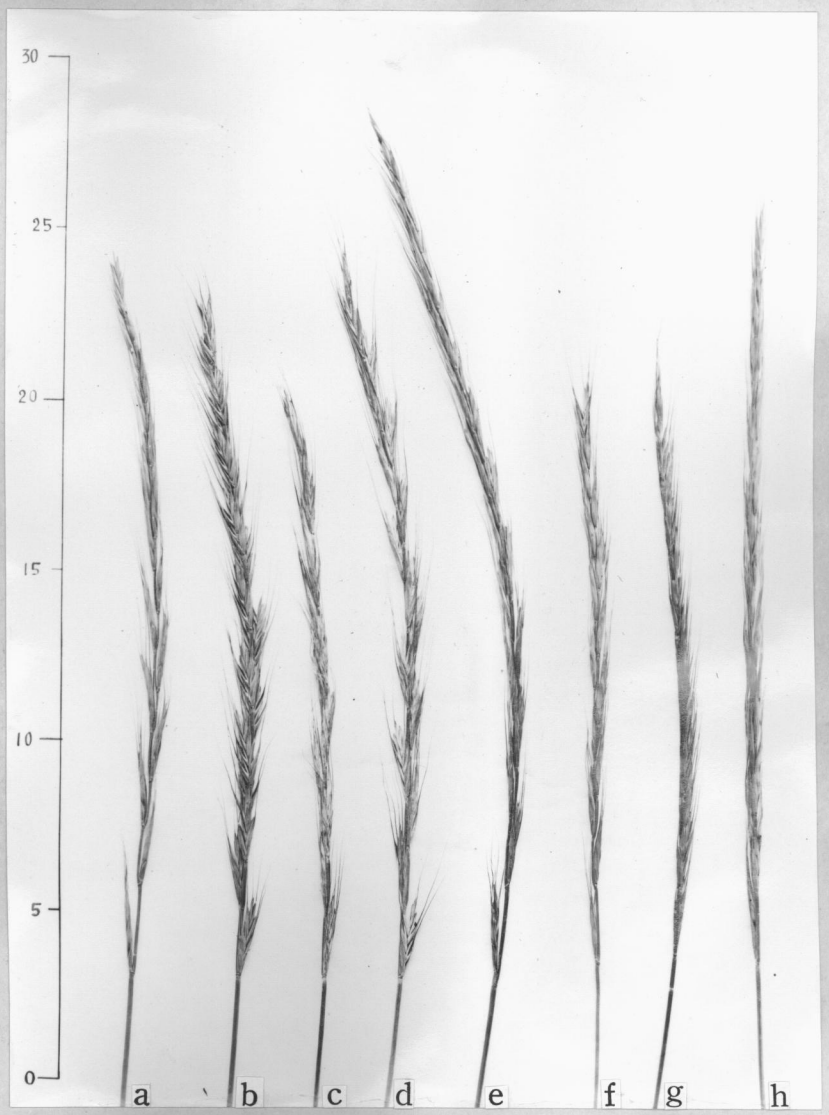
Chromosome pairing	No. of cells observed	
$12_{II} + 11_{I}$	1	
$1_{III} + 11_{II} + 10_{I}$	1	2 (7.1%)
$13_{II} + 9_{I}$	6	6 (21.4)
$14_{II} + 7_{I}$	16	
$1_{III} + 13_{II} + 6_{I}$	1	18 (64.4)
$1_{IV} + 12_{II} + 7_{I}$	1	
$15_{II} + 5_{I}$	2	2 (7.1)
Total	28	

vicinity of Misima, the first natural hybrid was found in Umena, south of Misima, in 1956. On an about 500m^2 area that was selected for detailed observation, Ag. humidum was found in the paddy fields in a high frequency. On the other hand the common type of Ag. tsukushiense was only found on the foot-paths and borders of irrigation ditches of the paddy field. Therefore, the ecological relationship of those two species was allopatric. Natural hybrids were observed mainly in Umena and, in addition, in Daiba, Naka and Sawaji, environs of Misima, where both species are found.

During 1956 - 1962 a total of 34 clones of natural hybrids were found on the foot-paths and borders of irrigation ditches or rarely in the paddy fields in those areas. Morphological characters of the natural hybrids are intermediate between the parental species or more closely resembling Ag. tsukushiense (Fig. 11). The hybrid in the natural habitats was recognized easily by its upright spikes, due to high sterility. Table 14 shows several quantitative characters of a natural hybrid (H_1) and the parental species growing near it in Umena. Of the 14 characters observed, five were intermediate and three were the same as in Ag. tsukushiense. Seed-setting of the natural hybrid clones was examined in the natural conditions as given in Table 15. Very low seed fertility was the rule; it varied from 0 to 1.5 per cent according to different clones and years.

The artificial hybrids were very easily obtained in 1956 as described previously, and the 17 F_1 plants were vigorous but completely sterile on the male side and highly sterile on the female side. Spikelets of the artificial hybrid and the parents are shown in Fig. 12. These plants were very similar to the natural hybrids. Chromosome

Fig. 11. Spikes of several natural hybrids and an artificial hybrid between Ag. humidum and the common type of Ag. tsukushiense



a. natural hybrid (H_1)
b. " (H_2)
c. " (H_6)
d. " (H_7)
e. " (H_8)
f. " (H_9)
g. " (H_{12})
h. artificial hybrid (5751)

Table 14. Comparison of quantitative characters of Ag. humidum, Ag. tsukushiense var. transiens and a natural hybrid (H_1) between the two species in Umena, Misima (1957)

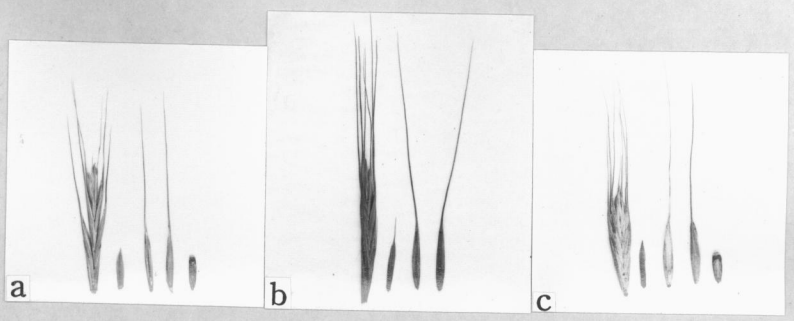
Characters \ Strains	Ag. humidum	Ag. tsukushiense	Natural hybrid (H_1)
Plant height (cm)	38.7	59.2	49.1
No. of tillers	80.0	22.0	25.7
Length of the first internode from top (cm)	21 9	34.8	28.4
Flag leaf Length (cm)	7.1	18.0	7.1
Flag leaf Width (cm)	-	1.1	0.7
Length of spike (cm)	13.4	25.4	21.0
No. of spikelets	10.2	23.0	16.0
No. of florets per spikelet	4.7	7.6	6.2
Empty glume Length (cm)	0.82	0.80	0.81
Empty glume Width (cm)	0.13	0.12	0.14
Outer glume with awn Length (cm)	2.76	3.23	3.60
Outer glume with awn Width (cm)	0.13	0.15	0.16
Inner glume Length (cm)	0.84	0.84	0.96
Inner glume Width (cm)	0.13	0.12	0.16
Seed Length (cm)	0.52	0.49	
Seed Width (cm)	0.11	0.13	(sterile)
Seed Weight (mg)	4.2	2.3	

Table 15. Seed fertility of the natural hybrids between Ag. humidum and the common type of Ag. tsukushiense at natural habitats in Misima

Strain	Locality	Year observed	Waxiness of plant	No. of spikes	No. of spikelets	No. of seeds	Seed fertility* (%)
H_{4-1}	Umena	1958	non-waxy	14	160	1	0.21
H_{4-2}	"	"	"	41	586	3	0.17
H_{6}	"	1959	waxy	11	159	1	0.21
H_{7}	"	1958	"	14	195	0	0
"	"	1962	"	53	773	3	0.13
H_{8}	"	1958	non-waxy	57	1,012	0	0
"	"	1962	"	10	133	1	0.25
H_{9}	"	1958	"	6	82	0	0
H_{10}	"	"	"	172	1,570	0	0
"	"	1959	"	140	1,417	3	0.07
"	"	1962	"	231	2,340	18	0.26
H_{11-1}	"	"	"	98	1,294	2	0.05
H_{11-2}	"	"	"	25	373	0	0
H_{15-1}	Sawaji	1958	"	12	189	0	0
H_{15-2}	"	"	"	21	337	0	0
H_{16}	Umena	"	"	21	253	0	0
"	"	1959	"	7	125	1	0.27
H_{20}	"	"	"	17	218	6	0.92
H_{29}	Naka	1962	"	50	830	0	0
H_{36-1}	Daiba	1959	"	21	347	15	1.44
H_{36-2}	"	"	"	6	88	4	1.52
H_{43}	Umena	1962	waxy	33	390	1	0.09
H_{44}	"	"	"	32	375	0	0
H_{45}	"	"	non-waxy	58	766	10	0.44
H_{46}	"	"	"	7	237	4	0.56
H_{47}	"	"	"	8	106	0	0
H_{48}	"	"	"	52	595	0	0
H_{49}	"	"	"	58	945	0	0
H_{50}	"	"	"	134	2,082	3	0.05
H_{52}	"	"	"	47	786	3	0.13
H_{53}	"	"	waxy	28	372	0	0

* seed fertility (%) = (No. of seed/No. of spikelet × 3) × 100

Fig. 12. Spikelet, empty glume, inside of outer glume (palea), outer glume and seed (from left to right) of Ag. humidum, an artificial hybrid (5751) and the common type of Ag. tsukushiense



a. Ag. humidum No. 1
b. An artificial hybrid (5751)
c. The common type of Ag. tsukushiense No. 2

pairing in meiosis of the artificial and the natural hybrids showed mostly 21_{II} (Table 16 and Fig. 9). As to chromosome pairing, no difference was found between the artificial and the natural hybrids.

b) Sympatric case --- Natural hybrids between Ag. humidum and the early ecotype of Ag. tsukushiense

The early ecotype of Ag. tsukushiense is often found in the paddy fields where Ag. humidum grows (Sakamoto 1961). Sympatric swarms of both species in the paddy fields were found in the valleys of the hilly neighborhood of Misima, particularly at Kamogahora, Misima. In order to estimate participation in the mixture of the two species, the number of clones of both should be counted in the swarm.

However, it is difficult to do so. In a more convenient and accurate way, the number of spikes and spikelets in the population was counted, using the one meter quadrat method in a paddy field where the hybrid plants were found. The result is given in Table 17. The number of spikelets of Ag. humidum was about the same as that of the early ecotype in the ratio of 1 in the former to 1.3 in the latter. This indicates that the effective number of spikelets contributing to natural interspecific hybridization between the two species is the same in the population.

In several swarms of both species, natural hybrid clones, namely 32 clones in a paddy field in 1958 and 40 clones in another paddy field in 1959, were found at Kamogahora. Since both parental species and the hybrids are perennial and clones are maintained year after year through subdivision of clones by culture practices used in rice planting time, the observed numbers do not necessarily indicate the frequency of annual occurrence of natural hybrids in the population.

On the other hand, 17 artificial hybrid plants between a strain

Table 16. Chromosome pairing of the artificial and natural hybrids and their parental species

Chromosome pairing	Ag. humidum No. 1	Artificial hybrid (5751)	Natural hybrids Misima (a-5)	Natural hybrids Fukuoka (a-7)	Ag. tsukushiense No. 2
21_{II}	114	35	56	73	132
1_{IV} $+19_{II}$	0	0	1	1	0
$20_{II}+2_{I}$	0	10	5	3	3
$1_{III}+19_{II}+1_{I}$	0	1	0	1	0
$19_{II}+4_{I}$	0	0	1	0	0
Total	114	46	63	78	135

Table 17. Frequency of spikes and spikelets in a natural swam of Ag. humidum and the early ecotype of Ag. tsukushiense var. transiens

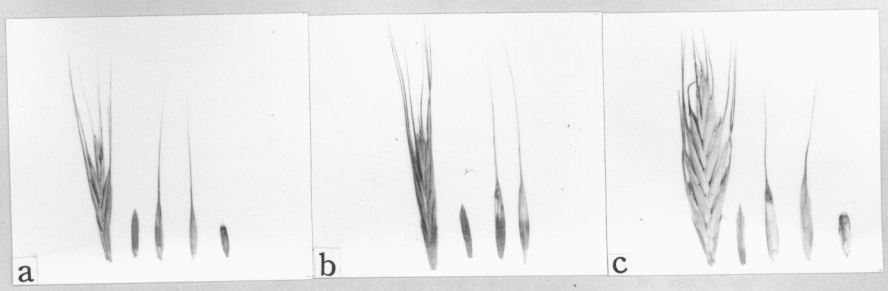
Species	Ag. humidum		Early ecotype of Ag. tsukushiense	
No. of quadrat	No. of spikes	No. of spikelets	No. of spikes	No. of spikelets
I	146	1,336	294	1,616
II	110	949	287	1,207
III	69	587	214	853
Total	325	2,872	795	3,676
Ratio	1	1	2.4	1.3

of Ag. humidum and a strain of the early ecotype of Ag. tsukushiense, both collected in Misima, were produced in 1958. They were completely male sterile, with non-dehiscent anthers, and no seeds were obtained in self- and open-pollination in the experimental field. The artificial and natural hybrids were morphologically very similar and resembled somewhat Ag. humidum as shown in Figs. 13 and 14. Table 18 shows the morphological characters and fertility of Ag. humidum, the early ecotype of Ag. tsukushiense and natural and artificial hybrids. A clone of a natural hybrid (a-48) (Table 18) was collected at Kamogahora in 1957. In 1958 the natural hybrids found in a paddy field showed a seed fertility of 0.4 per cent, namely 15 seeds from 1,209 spikelets of 32 clones. In the following year (1959) the fertility of natural hybrids in the other paddy field was examined and 0.2 per cent seed fertility was calculated having obtained 7 seeds from 1,431 spikelets of 40 clones. Since the anthers of natural hybrids were non-dehiscent at flowering time, all those seeds were assumed to have originated from backcrosses to Ag. humidum or to the early ecotype of Ag. tsukushiense. To acertain it, the 15 seeds obtained in 1958 were sown and five plants (cult. no. 5933) were obtained. A plant numbered 5933-1 died at an early stage of growth. The morphological characters and fertility of 5933 are shown in the lower half of Table 18. These plants showed about 0.3 - 31.2 per cent pollen fertility and 0 - 3.3 per cent open-pollinated seed fertility in the experimental field. The dehiscence of anthers, especially of 5933-2, -3 and -5, was good. A plant numbered 5933-2 showed 1.7 per cent seed fertility when self-pollinated and 5933-5 showed 28.6 per cent seed fertility when backcrossed to the early ecotype. Judging from the length of spike, number of spikelets,

Fig. 13. Spikes of natural and artificial hybrids and parental species, Ag. humidum and the early ecotype of Ag. tsukushiense

a. Early ecotype of Ag. tsukushiense
b. An artificial hybrid (5880)
c. A natural hybrid (H_{31})
d. Ag. humidum

Fig. 14. Spikelet, empty glume, inside of outer glume (palea), outer glume and seed (from left to right) of Ag. humidum, an artificial hybrid and the early ecotype of Ag. tsukushiense



a. Ag. humidum
b. An artificial hybrid (5880)
c. Early ecotype of Ag. tsukushiense

Table 18. Several characters and fertility of Ag humidum, early ecotype of Ag. tsukushiense and hybrids

Strain	Plant form	Waxiness	Length of spike(cm)	No. of spikelets	Pollen fertility (%)	Anthers	Seed fertility (%)			
							Selfed	Opened	× Ag. humidum	× Early ecotype
Ag. humidum (58115)	erect	non-waxy	14.0	10.6	98.3	dehiscent	65.0	-	-	31.4
Ag. humidum (Kamogahora)	erect	non-waxy	14.2*	8.5*	-	dehiscent	-	-	-	-
Ag. tsukushiense (58107)	laying	waxy	11.1	10.0	85.9	dehiscent	76.2	-	11.1	-
Ag. tsukushiense (Kamogahora)	laying	waxy	7.3*	5.7*	-	dehiscent	-	-	-	-
Natural hybrids	half-erect	non-waxy	10.9*	7.6*	-	not dehiscent	-	0.4('58) 0.2('59)	-	-
A natural hybrid (a-48)	half-erect	non-waxy	11.7	8.8	0	not dehiscent	0	0.03	0	4.0
Artificial hybrids (5882)	half-erect	non-waxy	7.2	5.0	0	not dehiscent	0	0	-	-
5933** -2	half-erect	waxy	8.0	5.9	30.5	dehiscent	1.7	0.6	-	0
-3	erect	non-waxy	10.2	10.9	22.0	dehiscent	0	1.9	-	-
-4	half-erect	waxy	8.3	6.2	0.3	-	0	0	-	0
-5	laying	non-waxy	10.0	7.3	31.2	dehiscent	0	3.3	-	28.6

* Plants from seeds obtained from natural hybrids in 1958 ** Four plants from seeds obtained from natural hybrids in 1958

plant habit and waxiness as shown in Table 18, it is concluded that of these 4 plants, 5933-2, -4 and -5 might have been derived from a natural hybrid backcrossed to the early ecotype and 5933-3 from pollination by Ag. humidum pollen in the natural swarm. This suggests that introgression might have occurred in the natural swarms involving the two species.

Progenies of these four backcrossed plants were tested in the experimental field in 1962 and 1963. Waxiness and seed fertility of self- or open-pollinated progenies of four strains (cult nos. 6207, 6208, 6209 and 6210) obtained in 1962 from three backcrossed plants (5933-2, -3 and -5) are given in Table 19. This table also records the waxiness and the individual variation in seed fertility of 16 strains, derived from these four progenies through self- or open-pollination, which were grown in 1963. Plants of culture nos. 6207, 6208, 6209 and 6210 were obtained from self-pollination of 5933-2, open-pollination of 5933-2, open-pollination of 5933-3 and open-pollination of 5933-5, respectively. A plant numbered 5933-4 did not produce any seeds either in self- or open-pollination. Cult. nos. 6207 and 6208 showed very low seed fertility. Three plants of 6209 were completely sterile. As to morphological characteristics these three plants were of Ag. humidum type, while 6207, 6208 and 6210 were similar to the early ecotype of Ag. tsukushiense. Of 13 plants of 6210, two plants were waxy and the rest were non-waxy. This suggests that 5933-5, the parent of 6210, was heterozygous and that non-waxy was dominant over waxy as also observed in F_1 plants. Five individuals of 6210 were completely sterile even in open-pollination. Plant numbered 6210-7 showed relatively high seed

Table 19. Waxiness and seed fertility of self- or open-pollinated progenies from three backcrossed plants (5933-2, -4 and -5). Detailed explanation in text.

Cult. number	Waxiness	Seed fertility (%) self	Seed fertility (%) open		Cult. number	Waxiness* w	nw	m	Seed fertility (%)* self-pollinated 0 - 20	20 - 40	40 - 60	60 - 80	80 - 100	Seed fertility (%)* open-pollinated 0 - 20	20 - 40	40 - 60	60 - 80	80 - 100
6207	w	3.0	0	self →	6326	2	0	0		1						1		
6208	w	0	6.6	open →	6327	9	0	0	8					8	1			
6209- 1	nw	0	0															
6209- 2	nw	0	0															
6209- 3	nw	0	0															
6210- 1	nw	-	0															
6210- 2	nw	16.1	-	self →	6328	0	5	0	4					3	1	1		
6210- 3	w	10.3	18.8	self →	6329	6	0	0	4					1	1	2	1	
"				open →	6330	10	3	0	8	4	1			4	2	2	4	1
6210- 4	w	38.9	35.5	self →	6331	24	0	0	13	4	1	2		12	6	1	4	1
"				open →	6332	24	0	0	16	2	3	1		7	8	4	3	1
6210- 5	nw	0	0															
6210- 6	nw	2.8	12.7	self →	6333	0	1	0	1					1				
"				open →	6334	1	12	1	6	3	1			9	1	2	1	1
6210- 7	nw	45.8	68.5	self →	6335	0	15	0	9	3	2	1		2	2	9	2	
"				open →	6336	0	27	0	13	10	3			4	4	10	9	
6210- 8	nw	0	0															
6210- 9	nw	25.0	13.6	self →	6337	0	2	0	2					2				
				open →	6338	0	4	0	2	1				2		1		
6210-10	nw	0	0															

Cult. number	Waxiness	Seed fertility (%) self	Seed fertility (%) open		Cult. number	Waxiness* w	Waxiness* nw	Waxiness* m	Seed fertility (%)* self-pollinated 0	20	40	60	80	100	Seed fertility (%)* open-pollinated 0	20	40	60	80	100
6210-11	nw	2.0	4.0	self →	6339	0	2	0	1						1					1
"				open →	6340	0	2	0								1				
6210-12	nw	0	0.9	open →	6341	0	1	0			1							1		
6210-13	nw	-	0																	

w: waxy, nw: non-waxy, m: mosaic * Number of plants observed

fertility, of 46 per cent in self- and 69 per cent in open-pollination.

Sixteen strains of self- or open-pollinated progenies from 6207, 6208 and 6210 (cult. nos. 6326 - 6341 in Table 19) cultivated in 1963 were morphologically very similar to the early ecotype of Ag. tsukushiense. Waxiness or lack of it of these strains shows that they are homozygous in this particular character except two strains, cult. nos. 6330 and 6334, being open-pollinated progenies.

The variation pattern of self- and open-pollinated seed fertility of these strains shown in Table 19 indicates that (1) the restoration of fertility is much more evident than in the previous generation, (2) fertility of several individual plants was the same as that of the original parental species and (3) plants with very low self-pollinated seed fertility, 0 - 20 per cent, represent the majority of the population.

V. DISCUSSION

(I) Morphology and sterility of the artificial hybrids

General appearance of many characters of the artificial hybrids was intermediate between the parents or superior to those of the parents. For example, with respect to 16 characters of F_1 plants between Ag. humidum No. 1 and Ag. ciliare No. 5 (Table 6), it can be said that Ag. humidum dominates in two characters, Ag. ciliare in four, four characters are intermediate between the two parents, five characters are superior to those of both parents and one character is the same as in both parents. In Ag. humidum No. 1 × Ag. tsukushiense No. 2 (Table 6), Ag. humidum dominates only in one character, Ag. tsukushiense in six, two characters are intermediate, six characters are superior to those of both parents and one character is the same as in both parents. In these two hybrid combinations about one third of characters examined were superior to those of the parents indicating hybrid vigor of F_1 plants.

Godley (1951) compared 14 characters of the F_1 hybrid between Ag. junceum and Ag. pungens with those of the two parents. He observed that Ag. pungens dominates in six characters, Ag. junceum in three, and that five characters are intermediate between the two parents. He also observed another hybrid between Ag. repens and Ag. junceum. Of the 14 characters studied, Ag. junceum dominates in four, Ag. repens in one, five characters are intermediate, two show an increase as compared with either parental species, and one shows a decrease.

Both anthers and ovaries of all F_1 hybrids were quite normal

in development, but complete pollen sterility and very high ovule sterility were the rule in all combinations of interspecific hybrids examined, even in those between two species with the same chromosome numbers, such as Ag. humidum No. 1 × Ag. tsukushiense No. 2. All anthers were non-dehiscent and all pollen grains were completely abortive. This type of sterility has been considered to be mainly due to cryptic structural hybridity of chromosomes of the parental species (Stebbins 1945), a phenomenon observed in many interspecific and intergeneric hybrids among the tribe Triticeae. According to Stebbins, Valencia and Valencia (1946), the intergeneric F_1 hybrid between Elymus glaucus and Sitanion jubatum is a typical case of chromosomal sterility due to cryptic structural hybridity. If the sterility of the F_1 is chromosomal, the amphidiploid induced by colchicine through doubling of chromosome number should have greatly increased fertility. This was clearly demonstrated by Stebbins and his coworkers in their interspecific and intergeneric hybrids in the tribe Triticeae (Stebbins, Valencia and Valencia 1946; others). The colchicine treatment has not been applied to the present F_1 hybrids but it is expected that with the doubling of chromosomes fertility would be increased. No further experimental approaches to elucidate the cause or the mechanism of this type of sterility have been made. However, as described in the previous chapter, rather quick restoration of seed fertility was found in the backcrossed plants and their progenies in the sympatric natural hybrid between Ag. humidum and the early ecotype of Ag. tsukushiense, both hexaploid Japanese species. This indicates that the mechanism of sterility in this particular interspecific hybrid might not be highly complicated.

High sterility in the interspecific F_1 hybrids observed in the

present study gives an important experimental basis for the estimation of the species as systematical entities in genus Agropyron. Any cross combination where two species were involved showed high sterility irrespective of the same chromosome numbers or genome homology of the species crossed. This provides a good experimental criterion for determining the species boundaries in Agropyron. In comparison with the concept of species based on experiments of Clausen, Keck and Hiesey (1939), the Japanese and Nepalese species would correspond to their distinct cenospecies. This type of relationship was reported also for the New Zealand Agropyron species by Conner (1956).

(II) Genomic relationships among species

The degree of chromosome pairing in interspecific or intergeneric hybrids can be used as an index of chromosome homology and species relationships. A higher amount of chromosome irregularities in hybrids indicates a relatively more distant relationship between the parents. Therefore, the number of bivalents in meiotic cells of interspecific hybrids is one of the best bases for the determination of the genomic relationships between the parental species. This has been clearly recognized as valid for several genera of the tribe Triticeae, such as Triticum and Aegilops (Lilienfeld 1951; Kihara 1954).

The species used in this study are tetraploid and hexaploid, with two sets of seven chromosomes making up the gametic complement of 14 chromosomes in the former and with three sets of seven chromosomes making up the gametic complement of 21 chromosomes in the latter. Since all the chromosomes are very similar in their size, no direct

distinction is possible, and, therefore, only indirect evidences have been used in determining the nature of pairing. For this purpose, multivalents observed in F_1 hybrids such as pentavalents, quadrivalents, and trivalents, were considered conveniently as two bivalents + one univalent, two bivalents, and one bivalent + one univalent, respectively. Frequency of bivalents according to this method is given in the second column for each hybrid combination in Tables 7, 8, 9 and 10. The numbers in parentheses indicate the percentage of cells having different bivalent numbers.

Range and mode of bivalent pairing, and average chromosome pairing in all interspecific hybrids are listed in Table 20. Moreover, frequency distribution of bivalent pairing is diagrammatically presented in Fig. 15 for Japanese 4x × Japanese 4x, Japanese 4x × Nepalese 4x and Japanese 4x × American 4x; in Fig. 16 for Japanese 4x × Japanese 6x; and in Fig. 17 for Nepalese 4x × Japanese 6x, respectively.

In Japanese 4x × Japanese 4x narrower variation of bivalent pairing (10_{II} - 14_{II}) with higher mode at 14 was obtained than in Japanese 4x × Nepalese 4x (Table 20). On the contrary, Japanese 4x × American 4x resulted in a very wide variation in bivalent formation, 2_{II} - 9_{II} with a very low mode at 5.

In pentaploid hybrids, a clear difference in the range of bivalent formation, mode and average number of bivalents were found between Japanese 4x × Japanese 6x and Nepalese 4x × Japanese 6x hybrids. A narrower range of bivalent formation (7_{II} - 16_{II}) with higher mode at 13 or 14 and higher average number of bivalents (12.2 - 13.2) was observed in the former compared with 3_{II} - 15_{II} with the

Table 20. Chromosome pairing in interspecific F_1 hybrids of *Agropyron* species

Cross combination (♀×♂) (cult. no.)	No. of cells examined	Range of bivalents	Mode of bivalents	Average chromosome pairing V	IV	III	II	I
Japanese 4x × Japanese 4x								
Ag. ciliare × *Ag. yezoense* (R170)	45	10 - 14	14		0.356	0.200	11.289	2.289
Japanese 4x × Nepalese 4x								
Ag. ciliare × *Ag. semicostatum* (5731)	408	5 - 14	12	0.002	0.135	0.150	11.336	4.196
Ag. semicostatum × *Ag. yezoense* (5743)	69	10 - 14	12		0.043	0.043	12.261	3.174
Japanese 4x × American 4x								
Ag. ciliare × *Ag. trachycaulum* (5733)	325	2 - 9	5		0.015	0.065	5.329	17.095
Japanese 4x × Japanese 6x								
Ag. tsukushiense × *Ag. ciliare* (5734)	107	9 - 15	14		0.056	0.075	13.056	8.439
Ag. tsukushiense × *Ag. yezoense* (5736)	87	7 - 15	14		0.149	0.333	12.241	8.805
Ag. yezoense × *Ag. tsukushiense* (5742)	55	13 - 15	14	0.036	0.182	0.291	12.600	8.010
Ag. gmelini (Japan) × *Ag. tsukushiense* (5888)	50	11 - 15	13		0.180	0.160	12.280	8.840
Ag. humidum × *Ag. ciliare* (5752)	53	12 - 16	14		0.094	0.019	13.170	6.453
Nepalese 4x × Japanese 6x								
Ag. tsukushiense × *Ag. semicostatum* (5737)	155	6 - 14	11		0.110	0.161	9.800	14.052
Ag. humidum × *Ag. semicostatum* (5886)	47	8 - 14	10				10.660	13.617
Ag. gmelini (Nepal) × *Ag. tsukushiense* (5884)	97	3 - 15	12		0.113	0.113	11.299	11.567
Ag. gmelini (Nepal) × *Ag. humidum* (5885)	25	9 - 15	11		0.040	0.080	11.920	10.760
Japanese 6x × Japanese 6x								
Ag. humidum × *Ag. tsukushiense* (5751)	46	20 - 21	21			0.022	20.739	0.457

Fig. 15. Frequency distribution of bivalents at MI in PMCs of F_1 hybrids: Japanese 4x × Japanese 4x, Japanese 4x × Nepalese 4x and Japanese 4x × American 4x.

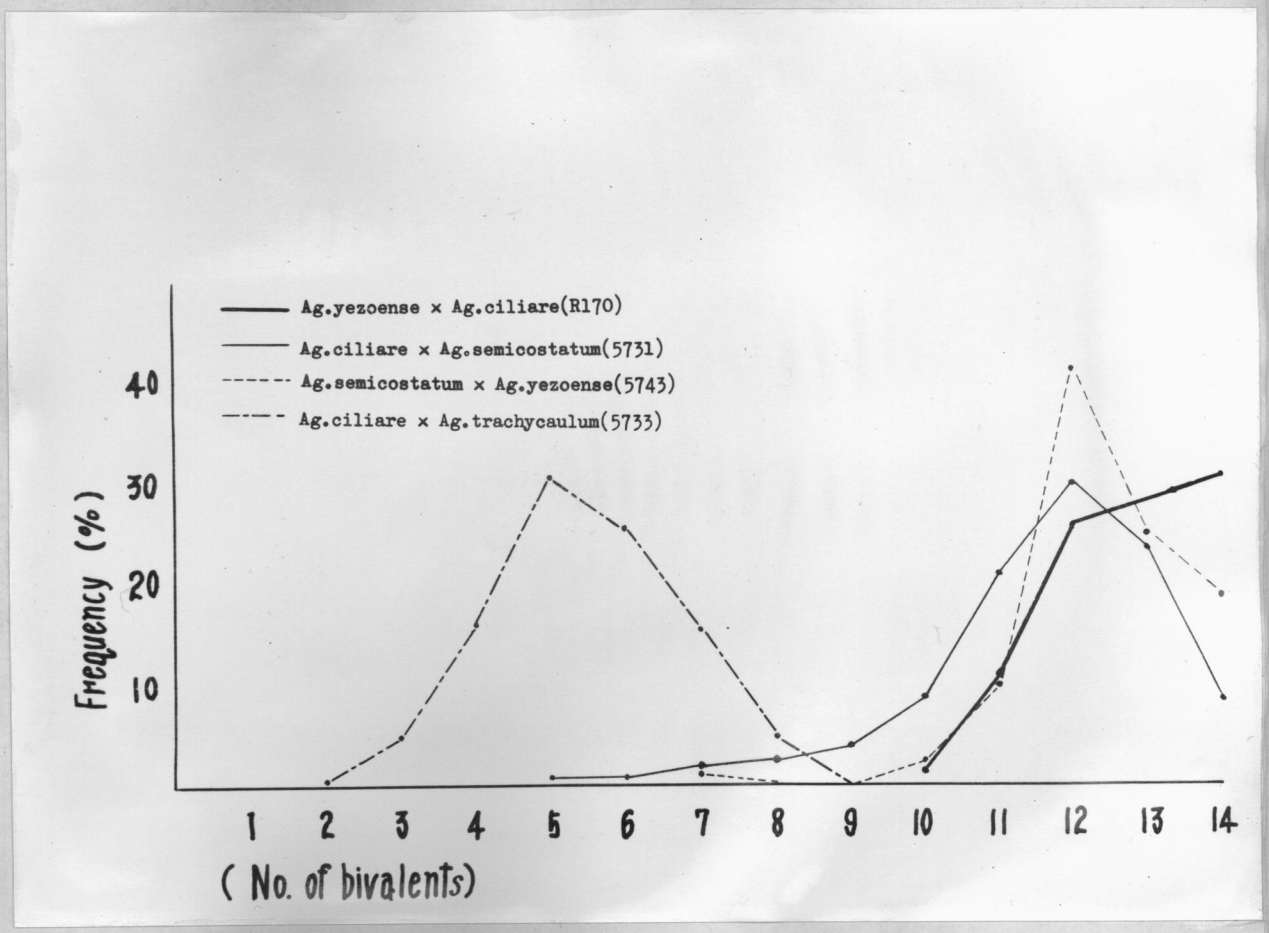


Fig. 16. Frequency distribution of bivalents at MI in PMCs of F_1 hybrids: Japanese 4x × Japanese 6x.

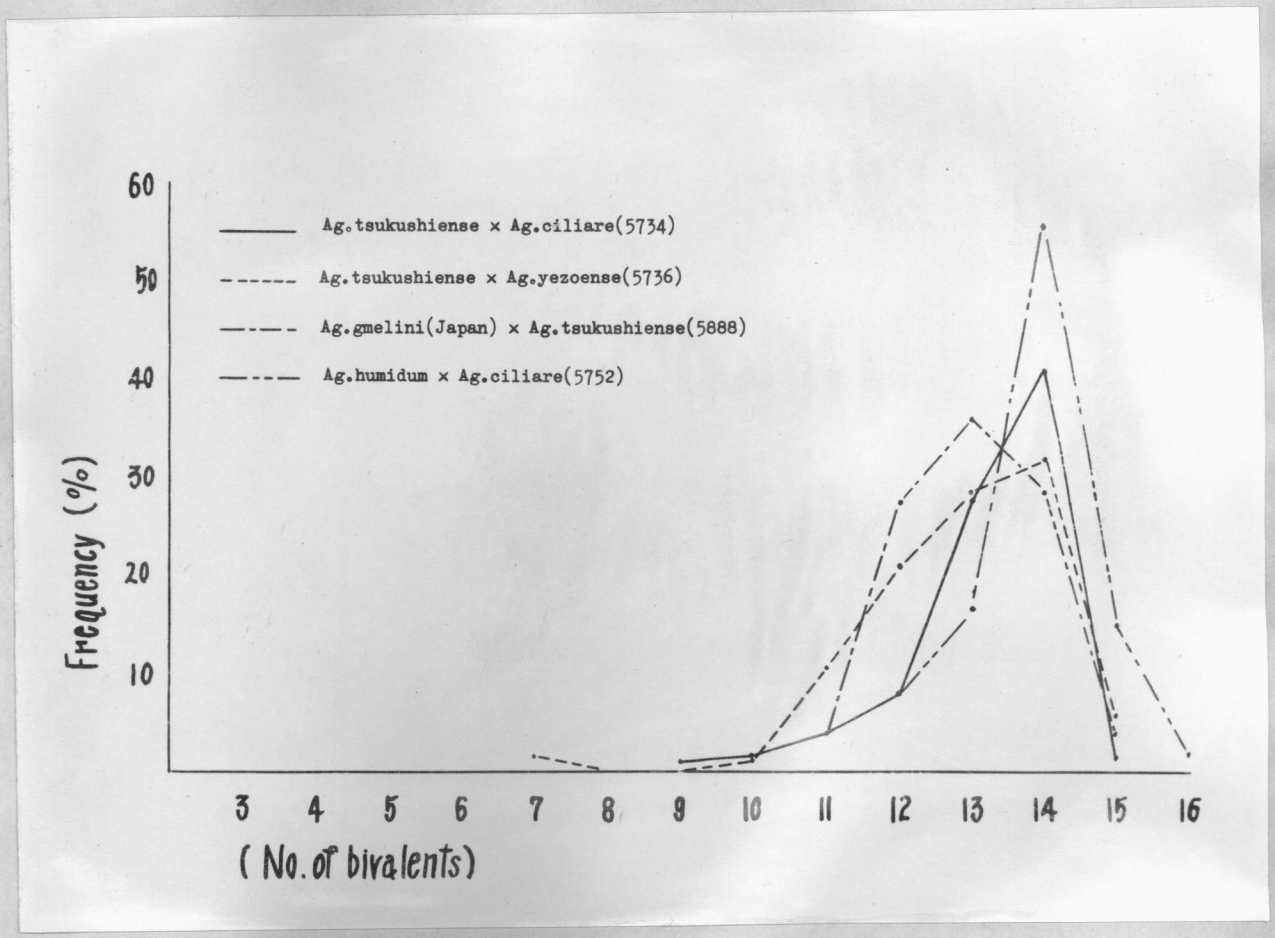
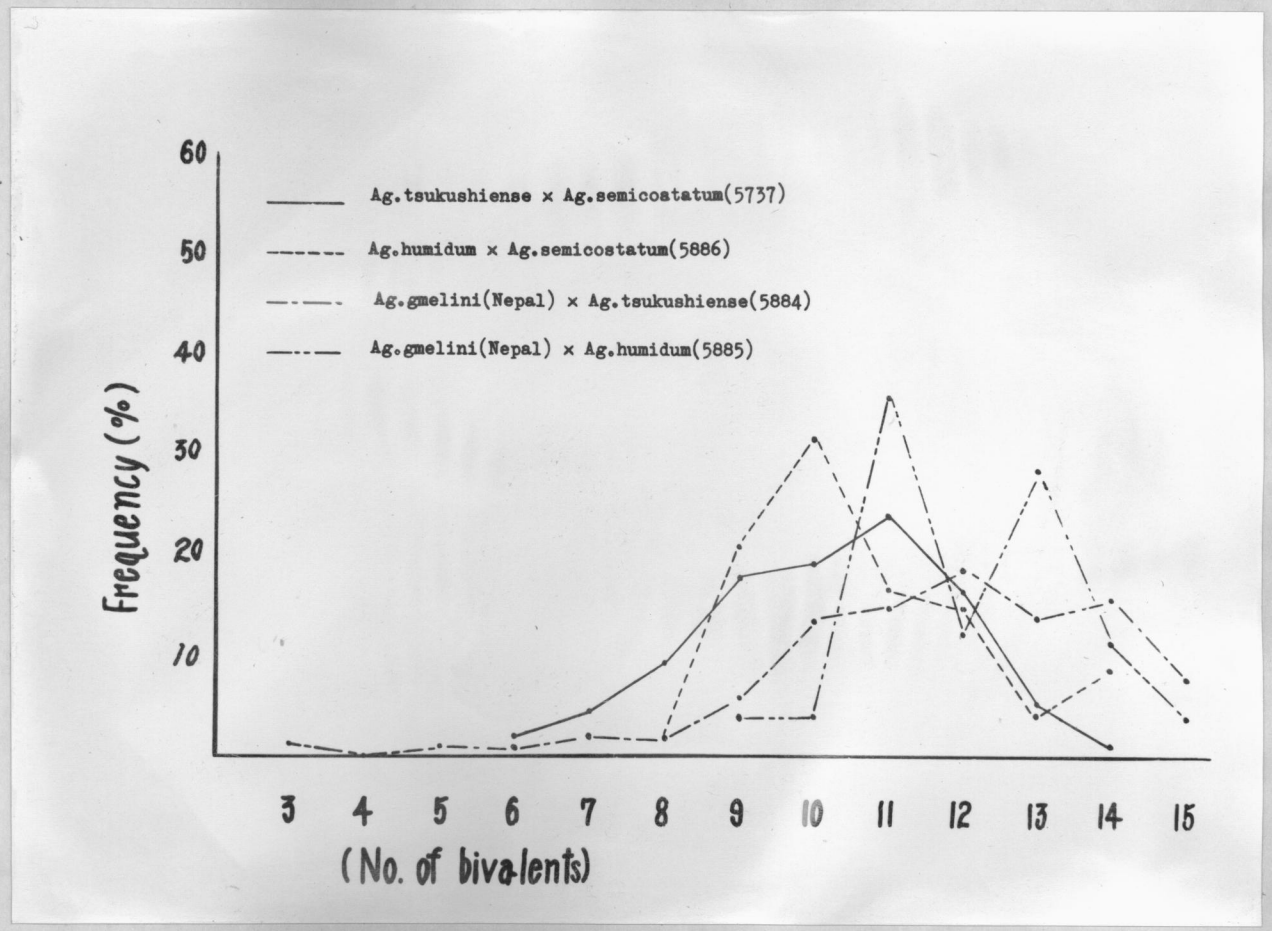


Fig. 17. Frequency distribution of bivalents at MI in PMCs of F_1 hybrids: Nepalese 4x × Japanese 6x.



mode at 10 or 12 and 9.8 - 12.0 of the latter hybrid combination, as shown in Table 20 and in Figs. 16 and 17.

In the hexaploid hybrid, 21_{II} in the majority of the meiotic cells were observed with a very small range of bivalent pairing, 20_{II} - 21_{II}, with the mode at 21, and 20.7 as the average number of bivalents (Table 20 and Fig. 8).

Chromosome pairing of the interspecific F_1 hybrids in Japanese Agropyron was first observed by Matsumura (1941, 1942, 1948) in Ag. ciliare × Ag. tsukushiense var. transiens and Ag. humidum × Ag. tsukushiense var. transiens. According to Matsumura, the range and the mode of bivalents in the former combination were 10_{II} - 14_{II} and 14, respectively. In Ag. humidum × Ag. tsukushiense hybrid, he observed many multivalents and univalents in meiosis but he could not analyze them in detail mainly due to the abnormal development of PMCs in a weak hybrid plant. From these observations, Matsumura (1948) has proposed the following genome formulae for his three species:

Ag. ciliare	II KK
Ag. humidum	II ?? ??
Ag. tsukushiense	II KK LL

He assumed that Ag. tsukushiense has two genomes, I and K, in common with Ag. ciliare and a third genome, L, but that Ag. humidum shares only one genome, I, with the other two species and has two other genomes which are different from K and L genomes.

From the results of the present study, the genome formulae for each species could be determined as shown in Table 21, applying Matsumura's three genome symbols, I, K and L, but some differentiation in each is indicated by the occurrence of quadrivalent and trivalent associations in the F_1 hybrid Ag. yezoense × Ag. ciliare No. 1

Table 21. Species, chromosome numbers and genome formulae of Japanese and Nepalese Agropyron

Species	2n	Genomes
Japanese species:		
Ag. repens P. Beauv.*	42	-
Ag. ciliare var. minus Ohwi	28	II KK
Ag. ciliare var. pilosum Honda	28	II KK
Ag. gmelini var. tenuisetum Ohwi	28	II KK
Ag. caninum L*	28	-
Ag. yezoense Honda var. yezoense	28	II KK
Ag. yezoense var. tashiroi Ohwi	-	-
Ag. humidum Ohwi et Sakamoto	42	II KK LL
Ag. tsukushiense var. transiens Ohwi	42	II KK LL
Ag. tsukushiense var. tsukushiense (Honda)	-	-
Ag. × hatusimae Ohwi**	42	II KK LL
Ag. × nakasimae Ohwi***	35	II KK L
Nepalese species:		
Ag. gmelini Scribn. et Smith	28	$I^{N}I^{N}$ $K^{N}K^{N}$
Ag. semicostatum Nees	28	$I^{N}I^{N}$ $K^{N}K^{N}$

* naturalized in Japan ** natural hybrid between Ag. humidum and Ag. tsukushiense *** natural hybrid between Ag. tsukushiense and Ag. ciliare

Although no direct data concerning the genomic affinity between Japanese Ag. gmelini and Ag. ciliare or Ag. yezoense have been available, the comparison of chromosome pairing of Ag. gmelini (Japan) × Ag. tsukushiense No. 2 with that of Ag. tsukushiense × Ag. ciliare No. 1 and Ag. tsukushiense No. 1 × Ag. yezoense indicates indirectly the existence of I and K genomes in Japanese Ag. gmelini. From the chromosome pairing of pentaploid F_1 hybrids between Japanese 4x × Japanese 6x, the genomic composition of two hexaploid species, Ag. humidum and Ag. tsukushiense, is II KK LL. Low frequency of multivalent association and existence of 6.5 - 8.8 univalents on the average in the pentaploid hybrid, Japanese 4x × Japanese 6x, suggest (1) a slight genomic differentiation in I and K genomes among the Japanese tetraploid species, and (2) no genomic homology between the third genome, L, and genomes I and K. No genomic differentiation was observed between the three genomes of Ag. humidum and those of Ag. tsukushiense, from the observation of tightly paired 21 bivalents in the majority of cells of Ag. humidum No. 1 × Ag. tsukushiense No. 2, as shown in Table 11 and in Fig. 9.

The genome structure of polyploid species has been estimated from chromosome pairing at MI of interspecific or intergeneric hybrids involving the species in question. However, this method of genome determination is usually inconclusive, if no diploid species are available as analysers. In many cases it is almost impossible to distinguish autosyndetic from allosyndetic pairing, according to the type of polyploidy of the species involved, either auto-, autoallo-, segmental autoallo- or allopolyploidy. In doubtful cases polyhaploid plants provide critical information concerning the genome structure of polyploid species (Dewey 1961). In 1962 a polyhaploid plant of

Ag. tsukushiense var. transiens was found in a state of nature in a valley of the hilly neighborhood of Misima (Sakamoto 1964a). Chromosome pairing at MI of PMCs of the polyhaploid, the monosomic (this plant was obtained from a seed which was assumed to have been produced by pollination with the pollen of Ag. tsukushiense growing nearby) and Ag. tsukushiense No. 2 are shown in Table 22. No bivalents were found in 84 per cent of the polyhaploid cells and only one (loosely associated by a terminal chiasma) was observed in 14 per cent of cells examined. Average chromosome pairing per cell was $0.0_{IV} + 0.0_{III} + 0.2_{II} + 20.6_{I}$, which indicates very low frequency of multivalent formation. Chromosome pairing of the monosomic and of Ag. tsukushiense No. 2 was $20_{II} + 1_{I}$ and 21_{II}, respectively, in the majority of cells. From these results it is concluded that Ag. tsukushiense var. transiens is an allohexaploid whose genome constitution comprises three different genomes (Sakamoto 1964a). This conclusion critically supports the view of Matsumura and the present investigations.

From these points discussed above, it is concluded that the genome constitution of the examined Japanese Agropyron species is very similar; the genomes shared by Japanese tetraploid species are I and K and a third genome, L, is added in the hexaploid species.

The present observation of chromosome pairing in Ag. tsukushiense No. 1 × Ag. ciliare No. 1 is very similar to Matsumura's observations of this hybrid combination. However, very different results were obtained from Ag. humidum No. 1 × Ag. tsukushiense No. 2 from those of Matsumura. In his experiment he used Ag. humidum collected in Fukuoka-Ken, Kyushu, in just the same place where the present Ag. humidum No. 1 was found. But as the male parent he used Ag. tsukushiense var. transiens collected in Wakayama-Ken, while the present Ag.

Table 22. Chromosome pairing at MI of PMCs of the polyhaploid, the monosomic and Ag. tsukushiense No. 2

Chromosome pairing	No. of cells observed	%
Polyhaploids:		
21_I	369	83.5
$1_{II} + 19_I$	61	13.8
$1_{III} + 18_I$	2	0.5
$2_{II} + 17_I$	8	1.8
$1_{IV} + 17_I$	1	0.2
$3_{II} + 15_I$	1	0.2
Total	442	100.0
Monosomic (cult. no. 6348-2):		
$20_{II} + 1_I$	164	97.0
$19_{II} + 3_I$	5	3.0
Total	169	100.0
Ag. tsukushiense No. 2:		
21_{II}	132	97.8
$20_{II} + 2_I$	3	2.2
Total	135	100.0

tsukushiense No. 2 was collected in Misima, Shizuoka-Ken. Although his F_1 plant showed very weak growth, its morphological description agrees with my observation. No evidence of a clear difference in chromosome pairing between mine and Matsumura's F_1 could be found.

From the comparison of chromosome pairing between two Japanese 4x × Nepalese 4x hybrids and a Japanese 4x × Japanese 4x hybrid, the following results were obtained:

1) a wider range of bivalent association,
2) a lower mode of bivalent number,
3) a significantly lower frequency of quadrivalent and trivalent associations,
4) no significant difference in the average number of bivalents and
5) a little higher occurrence of univalents, 3.2 - 4.2 as compared with 2.3 for a Japanese 4x × Japanese 4x hybrid

Moreover, from the comparison of chromosome pairing between four Nepalese 4x × Japanese 6x hybrids and five Japanese 4x × Japanese 6x, the results as to the former combinations are as follows:

1) a wider range of bivalent associations,
2) a lower mode for the bivalents,
3) lower average number of bivalent in a cell,
4) no significant difference in multivalent associations between the former and the latter combinations, and
5) higher occurrence of univalents in the former, 10.8 - 14.1, than in the latter, 6.5 - 8.8

Judging from those results, the genome formulae of two Nepalese species, Ag. gmelini and Ag. semicostatum, could be designated as $I^NI^N\ K^NK^N$ which indicates basically homologous genomes to I and K found in the Japanese species (Table 21). Some chromosomal

differentiation must have taken place between I^N and K^N genomes and I and K which reduced the number of bivalent association in Japanese × Nepalese hybrid (Table 20).

No direct comparison of genomes between Japanese and Nepalese Ag. gmelini has been made. The genomes of Ag. gmelini, indigenous to Japan as well as to Nepal, are truly homologous except for a slight but definite reduction in pairing. This result was obtained indirectly through crosses of both Japanese and Nepalese Ag. gmelini with Ag. tsukushiense, i.e. Ag. gmelini (Japan) × Ag. tsukushiense No. 2 and Ag. gmelini (Nepal) × Ag. tsukushiense No. 2 in Table 20.

In Ag. ciliare No. 1 × Ag. trachycaulum, (1) very wide variation of bivalent formation, (2) low mode of bivalent number, (3) very slight occurrence of multivalents, and (4) many univalents, 17_I, were observed. These evidences suggest two possibilities: firstly that one of Ag. trachycaulum genomes is very different from I or K genome of Ag. ciliare and another is partially homologous with I or K genome, or, secondly, that autosyndesis of Ag. trachycaulum genomes takes place in F_1 hybrid. Further experiments are needed to clarify this point. Stebbins and Snyder (1956) observed a triploid hybrid between Ag. trachycaulum and a diploid North American Ag. spicatum, obtaining $7_{II} + 7_I$ or $1_{IV} + 5_{II} + 7_I$ in the majority of cells in the hybrid. This indicates that a genome essentially homologous to that of Ag. spicatum exists in the tetraploid Ag. trachycaulum. Stebbins and Pun (1953) assumed that the genome homologous to the spicatum genome is widely spread in the northern temperate regions.

Judging from the chromosome pairing in Ag. ciliare No. 1 × Ag. trachycaulum, I or K genome in Ag. ciliare is assumed to be different from but partially homologous with the spicatum genome.

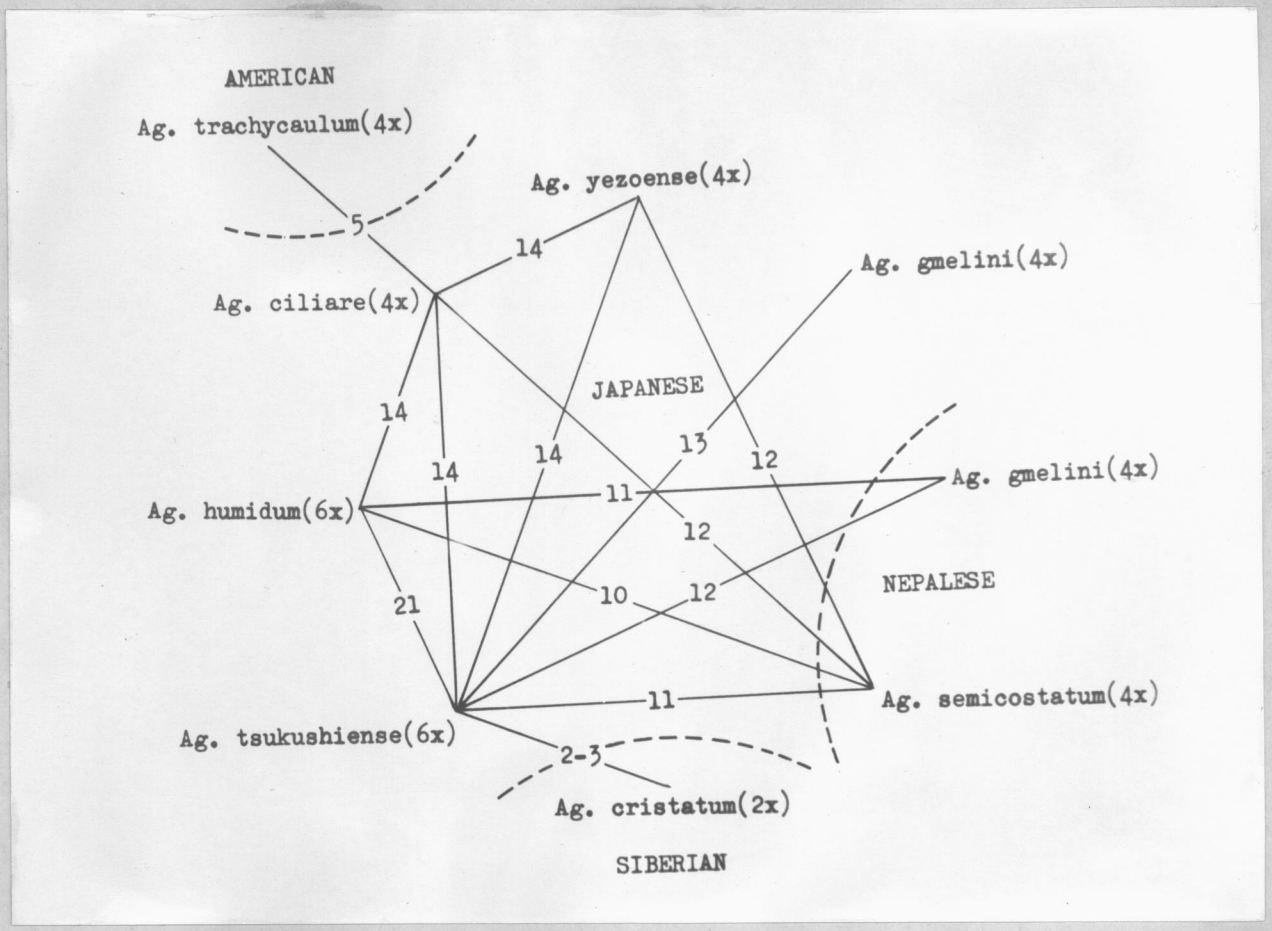
As will be discussed later, Ag. × hatusimae was identified as a natural hexaploid hybrid between Ag. humidum and Ag. tsukushiense var. transiens, and Ag. × nakasimae was also identified as a natural pentaploid hybrid between Ag. ciliare and Ag. tsukushiense var. transiens. Therefore, the genome formulae for these two natural hybrids are II KK LL for the former and II KK L for the latter as shown in Table 21.

(III) Relationships between genomic differentiation and geographical distribution

Hybrids between geographically isolated species are especially interesting because they afford the opportunity to study the effects of geographical isolation on species formation.

From the present results of cytological observations of the hybrids, the interspecific relationships of the present materials, five Japanese, two Nepalese and one American species is given in Fig. 18, in terms of the mode of bivalents in the hybrids. In this figure an information on the hybrid between Ag. tsukushiense and Ag. cristatum, a diploid Siberian species, is given after Matsumura (1942). In both tetraploid and pentaploid interspecific hybrids between Japanese and Nepalese species, the mode of bivalent formation was higher in Japanese × Japanese species hybrids than in Japanese × Nepalese hybrids and the variation range of bivalents was narrower in the former than in the latter. In addition to this, frequency of univalents at MI in the former hybrids was lower than in the latter, but in several cases, multivalent formation, on the contrary, was higher in the former than in the latter (Table 20). Those evidences lead to the conclusion that the two Nepalese species are closely related to the Japanese species but some degrees of genomic differentiation are indicated.

Fig. 18. Interspecific relationships among nine species of Japanese, Nepalese, American and Siberian Agropyron in terms of the mode of bivalent number in the hybrids



Therefore, the genomes of the two Nepalese species were designated as $I^{N}_{I}{}^{N}$ $K^{N}_{K}{}^{N}$ as discussed above.

According to Good (1953), Japan and Nepal belong to the same phyto-geographical region, the Sino-Japanese Region, which includes (1) the elevated area of the Sino-Himalayan-Tibetan mountains; (2) the rest of China except the South; and (3) the insular area, Japan. This view was supported by Kitamura (1955) after the taxonomical examination of the herbarium specimens of flowering plants collected by the members of the Japanese Scientific Expedition to Nepal Himalaya, 1952 - 1953. Kitamura found in Nepal many closely related species to those found in Japan. In the studies of Agropyron and related genera found in Nepal Himalaya, Matsumura, Sakamoto and Tateoka (1956) reported Ag. gmelini, Elymus dahuricus, El. sibiricus and Brachypodium sylvaticum which are also distributed in Japan.

Cytogenetical studies of Japanese and Nepalese Agropyron species provide experimental evidences of genomic differentiation owing to geographical isolation of long duration.

On the contrary, the chromosome pairing between Ag. ciliare No. 1 and Ag. trachycaulum suggests the existence of different or partially homologous genomes in an American tetraploid species which is distributed from Labrador to Alaska, south of the mountains of West Virginia, Missouri, New Mexico and California, and northern Mexico (Hitchcock 1951), i.e., in the Pacific and the Southern Atlantic North America Regions (Good 1953).

However, there are several cases showing a contrary situation from that of Agropyron hybrids. According to Sax (1935), Platanus occidentalis and P. orientalis must have been separated for thousands of generations, but the F_1 hybrid shows normal meiotic division and

fertility. Also, the F_1 hybrid between Asiatic and European species of Larix is fertile and the chiasma frequency of the hybrid is as high as that in the parental species. This behavior seems to indicate that such widely separated species sometimes do not undergo any fundamental genetic and structural changes in the chromosomes for very long time periods (Sax 1935). Stebbins (1950) considers that further studies of hybrids between widely allopatric species will probably reveal that the situation found in Platanus is not uncommon in the higher plants.

(IV) Natural hybridization in Japanese Agropyron

In the present study, two natural hybrids involving one tetraploid, Ag. ciliare, and two hexaploid species, Ag. humidum and Ag. tsukushiense, of Japanese Agropyron were reported. They are Ag. ciliare × Ag. tsukushiense and Ag. humidum × Ag. tsukushiense.

In the former pentaploid hybrid, natural seed setting was not observed in natural hybrid clones which have grown together with both parental species mainly on river dikes and in the hills of Misima (Fig. 10 and Table 12). It is assumed that in this hybrid combination introgressive hybridization could not occur in natural conditions. Natural hybrids have been collected in Fukuoka, Kyushu by Mr. K. Nakajima and were first classified as Ag. Mayebaranum var. Nakasimae (Ohwi) Ohwi or Ag. Nakasimae Ohwi (Ohwi 1942b). Ohwi's morphological descriptions of this form well agreed with the observation of the present materials. The correct binary name for this natural hybrid is Ag. × nakasimae Ohwi.

In the latter hexaploid natural hybrid, Ag. humidum × Ag. tsukushiense, two cases were found in Misima; i.e., one allopatric and the other sympatric. In the allopatric case Ag. humidum and the

common type of Ag. tsukushiense var. transiens, occurring in nature in different ecological habitats, were involved, while, in the sympatric case Ag. humidum and the early ecotype of Ag. tsukushiense var. transiens, both growing in fallow paddy fields, were involved in natural hybridization.

In the allopatric case very low seed fertility was the rule; it varied from 0 to 1.5 per cent according to different clones and years (Table 15). This indicates that spontaneous backcrossing could occur sometimes under natural conditions. For example, clone no. H_4-2 in Table 15 showed a seed fertility of 0.17 per cent in 1958 and three seeds were obtained. From these seeds one plant (cult. no. 5932b) whose characters were mostly very similar to those of the common type of Ag. tsukushiense, was grown in the experimental field in 1959. This plant had relatively high pollen and seed fertilities, namely:

Pollen fertility		62.74%
Seed fertility	self-pollinated	65.93%
	open-pollinated	77.72%

It is assumed that 5932b might be the offspring of the backcross to Ag. tsukushiense as the pollen parent.

Those natural hybrids have been found in Fukuoka-Ken, Kyushu (Nakajima 1952) and the hybrid specimens have been referred by Ohwi (1942b) to Ag. Hatusimae Ohwi or Ag. Mayebaranum var. intermedium Hatusima (Ohwi 1953). Ohwi's morphological description agreed with that of the present materials which were identified by him. Mr. T. Osada of Fukuoka High School, kindly collected several hybrid strains in Fukuoka in 1955. They showed very similar characteristics as the Misima strains. The correct binary name for this natural hybrid is

Ag. × hatusimae Ohwi.

In the sympatric case, natural hybrids were found in the valleys of the hilly neighborhood of Misima, particularly at Kamogahora where both parental species were found in swarms in fallow paddy fields. Natural seed setting amounted to 0.2 - 0.4 per cent. These seeds are assumed to have been produced by pollination with the pollen of either Ag. humidum or the early ecotype of Ag. tsukushiense growing together with the hybrid plants. Morphological characters and fertility of four plants derived from these seeds indicated that of these four plants, three might have been derived from a natural hybrid backcrossed to the early ecotype of Ag. tsukushiense and one plant from pollination by Ag. humidum pollen in the natural swarm. The variation pattern of seed fertility of the progeny of these backcrossed plants (Table 19) showed rather quick restoration of seed fertility in several individuals but the majority of the population were plants with low self-pollinated seed fertility, 0 - 20 per cent.

Judging from the progeny tests of the backcrossed plants in the experimental field mentioned above, the following conclusion may be drawn. It is quite possible that introgression of characters such as waxiness from one parental species to the other might have occurred in the natural populations through backcrossing of the parental species to natural F_1 hybrids followed by segregation of the characters concerned. Rather quick restoration of fertility in the hybrid progenies might make the establishment in nature of hybrid swarms possible having intermediate characteristics of the two parental species. Since the species of Agropyron are perennial, natural F_1 hybrids, backcrossed plants and their later progenies would be easily propagated

vegetatively in the fallow paddy fields.

However, no introgressants have been found yet so far in the natural sympatric populations of Ag. humidum and the early ecotype of Ag. tsukushiense found at Kamogahora, Misima. High sterility of F_1 hybrids might be a major cause of the restriction of introgressive hybridization between these two species.

(V) Considerations on the speciation of Japanese Agropyron

Complete pollen sterility and very high ovule sterility were the rule in all interspecific hybrids examined, even when the parents had the same chromosome numbers and the same genomes. This sterility is an important basis from the viewpoint of the species concept of the genus. Three indigenous tetraploid species having the same genomes, Ag. ciliare, Ag. gmelini and Ag. yezoense, can be definitely distinguished by this experimental criterion in addition to the conventional taxonomic key characters. Also, two hexaploid species, Ag. humidum and Ag. tsukushiense, are critically differentiated by hybrid sterility. Although, as shown in Table 20, small amount of quadrivalents or trivalents were detected in all interspecific hybrids of 4x × 4x, 4x × 6x and 6x × 6x hybrids, the cause of the sterility was assumed to be mainly due to cryptic structural hybridity of the chromosomes of the parental species.

High sterility results in isolation barriers among the species of this genus restricting introgression of genes between them. This conclusion has been reached after my observation of natural pentaploid hybrids between Ag. ciliare and Ag. tsukushiense, and natural swarms of two hexaploid species, Ag. humidum and Ag. tsukushiense.

On the contrary, intraspecific hybrids within a species were fertile. As mentioned in Chapter II, Ag. ciliare occurs in two

varieties, var. minus and var. pilosum. The former is distinguishable from the latter by nodding spikes with pubescent outer glumes. A hybrid between the two varieties was fertile (Sakamoto, unpubl.). In nature intermediate forms with erect spikes and pubescent glumes or those bearing nodding spikes with non-pubescent glumes are found together with the typical minus and pilosum forms. The genetical differences between the two varieties might be controlled by several genes. A study is now in progress. Ag. yezoense var. tashiroi and Ag. tsukushiense var. tsukushiense are distinguished from Ag. yezoense var. yezoense and Ag. tsukushiense var. transiens, by the pubescent outer glume of the former two varieties. Although no experimental data are available, this character may be governed by one or two genes. As mentioned previously, two species, Ag. × hatusimae and Ag. × nakasimae, are natural interspecific hybrids.

Interecotypic F_1 and F_2 progenies between the common type and the early ecotype of Ag. tsukushiense var. transiens were fertile. Genetic differences between both types were demonstrated to be due to several major genes and polygene systems governing the quantitative characters. Cytological differentiation was also indicated by quadrivalent formation (14 per cent of the observed cells) in meiosis of F_1 (Sakamoto 1961).

Five indigenous species found in Japan are all polyploids, either tetra- or hexaploid. No diploid species has been found yet in Japan. Judging from the results of the present study mentioned in Chapter IV, these species are allopolyploids combining genetically different genomes, II and KK in tetraploid and II KK LL in hexaploid species. This was also confirmed by the observation of chromosome pairing at MI of a spontaneous polyhaploid plants (2n=21) of Ag. tsukushiense

var. transiens which was found in a valley of Misima in 1962 (Sakamoto 1964a). If the present species were originated from diploid ones, the three tetraploid species could have arisen from an ancestral tetraploid species produced by hybridization of two diploid species followed by doubling of chromosome numbers. because all tetraploid species possess the same two genomes, I and K. The two hexaploid species could have been produced from the tetraploid II KK species and a third diploid species with L genome.

However, there are no available data about the history of species formation at present. Most important would be to find the diploid ancestors that contributed to the formation of Japanese Agropyron species.

The origin center of the Asiatic Agropyron seems to be in China Proper in the central part of the Sino-Japanese Region measuring about 5,000,000km^2. Keng (1959) reported 49 indigenous Agropyron species as Roegneria and three as Eu-Agropyron in China including Taiwan, Mongolia and Tibet. Chromosome numbers of most of the species described in his book, except those distributing in Japan, have not been studied yet. It is strongly desired to collect those materials in order to find among them the diploid species which might have been the ancestors of Japanese Agropyron, and to examine cytogenetic and phylogenetic relationships between Chinese and Japanese species. Since all indigenous Japanese species are distributed not only in Japan but also in China, Korea, Manchuria or Siberia as shown in Table 2, they might have originated in the Asiatic Continent, probably in China. Japan is the eastern border of their distribution area facing the Pacific Ocean.

Intraspecific differentiation of the species seems to have

occurred in Japan, for example, Ag. yezoense var. tashiroi and Ag. tsukushiense var. tsukushiense might be such cases. These two varieties are found in very restricted areas in Japan and they differ from the representative variety of the species in several characters. More detailed exploration of each species in the natural habitat could provide the evidence of various degrees of intraspecific differentiation of Japanese species. As reported by Sakamoto (1961), the early ecotype of Ag. tsukushiense var. transiens was probably distributed in Japan rather recently. It could have been introduced to Japan from China together with seeds of Chinese milk-vetch or other forage crops. The same assumption was expressed for Ag. humidum by Ohwi and Sakamoto (1964).

Interspecific hybridization in the natural habitat where two species occur together, was observed in Ag. ciliare and Ag. tsukushiense, and in Ag. humidum and Ag. tsukushiense. Although no introgressants have been found yet in either case due to high sterility of F_1 hybrids causing sexual isolation, introgression of certain genes and restoration of fertility in the hybrid progenies, examined in the experimental field, between Ag. humidum and the early ecotype of Ag. tsukushiense, indicates the occurrence of restricted introgressive hybridization might have played an important role in the process of speciation in Japanese Agropyron

VI. CONCLUSION AND SUMMARY

Japanese Agropyron species were studied from cytogenetical point of view in order to analyse the variation patterns within species, which could provide experimental bases for distinguishing natural biosystematic units and for determining species relationships.

1. Morphological, physiological and ecological characteristics of five indigenous species, i.e., three tetraploids, Ag. ciliare, Ag. gmelini and Ag. yezoense, and two hexaploids, Ag. humidum and Ag. tsukushiense, were observed.

2. From morphological, physiological and cytogenetical studies of 14 different interspecific hybrid combinations among five Japanese, two Nepalese and an American species, the following conclusions are drawn:

1) Most of the interspecific F_1 plants are vigorous and in general the characters in F_1 are either intermediate between the parents or superior to those of the parents.

2) Complete pollen sterility and high ovule sterility were the rule in all combinations examined. The cause of sterility was assumed mainly to be due to cryptic structural hybridity of chromosomes of the parental species.

3) High sterility of interspecific F_1 hybrids observed in the present study provides an important experimental basis for the estimation of species as systematical entities in genus Agropyron. Japanese and Nepalese species seem to be distinct cenospecies.

4) From chromosome pairing in F_1 hybrids, the genome constitution of five Japanese and two Nepalese species was determined as shown in Table 21. It is concluded that the genome constitutions of the

Japanese species are very closely related with each other; two genomes, I and K, are shared by three Japanese tetraploid species, Ag. ciliare, Ag. gmelini and Ag. yezoense and a third genome, L, is added in two hexaploid species, Ag. humidum and Ag. tsukushiense. This was also confirmed by the cytological observation of chromosome pairing at MI of a polyhaploid plant of Ag. tsukushiense var transiens. The genome formula of two Nepalese species, Ag. gmelini and Ag. semicostatum, can be designated as $I^{N}I^{N}$ $K^{N}K^{N}$, the superscript N (Nepal) indicating basical homology to I and K genomes found in Japanese species. The genomes of Ag. gmelini, indigenous to Japan as well as to Nepal, are truly homologous except for a slight but definite reduction in pairing. This result was obtained indirectly through crosses of both Japanese and Nepalese Ag. gmelini with Ag. tsukushiense. An American tetraploid species, Ag. trachycaulum, seems to have somewhat different genomes from those of the Japanese species.

5) Cytogenetical studies between Japanese and Nepalese species provide experimental evidences of genomic differentiation by geographical isolation, lasting a long time, of closely related species which are distributed in the same phyto-geographical area, the Sino-Japanese Region.

3 Two cases of natural interspecific hybridization in Japanese Agropyron were observed in Misima. One is the pentaploid hybrid between Ag. ciliare and Ag. tsukushiense, and the other is the hexaploid hybrid between Ag. humidum and Ag. tsukushiense.

1) No seed setting was observed in the pentaploid hybrid due to extremely high sterility of both male and female organs of the hybrid. It is assumed that in this hybrid combination introgressive hybridization would not occur in natural conditions.

2) As to hexaploid natural hybrids, two cases were observed; i.e., one allopatric and the other sympatric. In the allopatric case Ag. humidum and the common type of Ag. tsukushiense were involved, while, in the sympatric case Ag. humidum and the early ecotype of Ag. tsukushiense. In both cases low seed setting was observed examining a considerable number of hybrid clones in natural conditions. From progeny tests of backcrossed plants obtained from the sympatric natural hybrids between Ag. humidum and the early ecotype of Ag. tsukushiense, the following conclusions are drawn: It is quite possible that introgression of characters, such as waxiness, from one species to the other might have occurred in the natural populations through backcrossing to the parental species of natural F_1 hybrids followed by segregation of the characters concerned. Rather quick restoration of fertility in the hybrid progenies makes establishment of hybrid swarms in a state of nature possible having intermingled characteristics of the parental species. However, so far no introgressants have been found yet in the natural sympatric populations of these two species. High sterility of F_1 hybrids might be a major cause of the restriction of introgression.

4. Several considerations on the speciation of Japanese Agropyron were discussed, based upon experimental evidences described in the present thesis.

VII. LITERATURE CITED

Avdulov, N. P. 1931. Karyosystematische Untersuchungen der Familie Gramineen. Bull. Appl. Bot., Genet. and Pl. Breed., Suppl. 44: 1-428.

Cauderon, Y. 1958. Étude cytogénétique des Agropyrum francais et de leurs hybrides avec les blés. Thèses présentées a la Faculté des Sciences de L'Université de Paris. pp. 161.

Clausen, J., D. D. Keck and W. M. Hiesey 1939. The concept of species based on experiment. Amer. Jour. Bot. 26: 103-106.

Conner, H. E. 1954. Studies in New Zealand Agropyron. Part I. Descriptions and distribution of species and infraspecific units. Part II. Breeding systems. New Zealand Jour. Sci. and Tech. Section B 35: 315-343.

Conner, H. E. 1956. Interspecific hybrids in New Zealand Agropyron. Evolution 10: 415-420.

Conner, H. E. 1957. Agropyron × wallii, a natural hybrid in New Zealand. Trans. Royal Soc. New Zealand 84: 757-760.

Conner, H. E. 1962. Studies in New Zealand Agropyron. Part III. Intraspecific hybrids in A. scabrum (R. Br.) Beauv. New Zealand Jour. Sci. 5: 99-115.

Dewey, D. R. 1961a. Hybrids between Agropyron repens and Agropyron desertorum. Jour. Hered. 52: 13-21.

Dewey, D. R. 1961b. Polyhaploids of crested wheatgrass. Crop. Sci. 1: 249-254.

Dewey, D. R. 1962. Morphology, fertility, and cytology of Agropyron repens × Agropyron desertorum F_2's. Amer. Jour. Bot. 49: 78-86.

Dewey, D. R. 1963. Cytology and morphology of a synthetic Agropyron trichophorum × Agropyron desertorum hybrid. Amer. Jour. Bot. 50: 552-562.

Franchet, A. and Savatier, L. 1879. Enumeratis Plantarum Japonicarum II: 184-186.

Godley, E. J. 1951. Two natural Agropyron hybrids occurring in the British Isles. Ann. Bot. 15: 535-544.

Good, R. 1953. The Geography of the Flowering Plants. Longmans, Green and Co., London, pp. 452.

Hartung, M. E. 1946. Chromosome numbers in Poa, Agropyron, and Elymus. Amer. Jour. Bot. 33: 516-531.

Heneen, W. K. 1962. Karyotype studies in Agropyron junceum, A. repens and their spontaneous hybrids. Hereditas 48: 471-502.

Heneen, W. K. 1963. Meiosis in the interspecific hybrid Elymus farctus × E. repens (=Agropyron junceum × A. repens). Hereditas 49: 107-118.

Hitchcock, A. S. 1951. Manual of the Grasses of the United States. U.S.D.A. Miscellaneous Pub. 200, pp. 1051.

Honda, M. 1927. Revisio Graminum Japoniae. 13(in Jap.). Bot. Mag. Tokyo 41: 377-389.

Honda, M. 1929. Nuntia ad Floram Japoniae. III. (in Jap.). Bot. Mag. Tokyo 43: 291-294.

Honda, M. 1930. Monographia Poacearum Japonicarum, Bambusoideis exclusis. Jour. Fac. Sci. Imp. Univ. Tokyo, Sect. III. Bot. III. Part I: 1-484.

Honda, M. Nihon-san Kahon-ka Shokubutsu Kôki (1) (in Jap.) Bot. Mag. Tokyo 45: 211-215.

Honda, M. 1935. Nuntia as Florum Japoniae. XXIV. Bot. Mag. Tokyo 49: 694-700.

Honda, M. 1936. Nuntia ad Florum Japoniae. XXVI. Bot. Mag. Tokyo 50: 389-392.

Hunziker, J. H. 1955. Artificial and natural hybrids in the Gramineae, Tribe Hordeae. VIII. Four hybrids of Agropyron and Elymus. Amer. Jour. Bot. 42: 459-467.

Keng, Yi-Li (Ed.) 1959. Flora Illustralis Plantarum Primarum Sinicarum. Gramineae (in Chinese). Scientific Book Pub., Peking, pp. 1181.

Kihara, H. 1954. Considerations on the evolution and distribution of Aegilops species based on the analyser-method. Cytologia 19: 336-357.

Kitamura, S. 1955. Flowering plants and ferns. In Fauna and Flora of Nepal Himalaya. Scientific Results of the Japanese Expedition to Nepal Himalaya, 1952-1953, Vol. I: 73-290.

Lilienfeld, F. A. 1951. Genome-analysis in Triticum and Aegilops. X. Concluding review. Cytologia 16: 101-123.

Makino, T. 1903. Observation on the Flora of Japan. Bot. Mag. Tokyo 17: 128.

Matsumura, J. 1905. Index Plantarum Japonicarum. II. p. 33.

Matsumura, S. 1941. Interspecific hybrids in Agropyron (in Jap.). Kagaku 11: 245-248.

Matsumura, S. 1942. Interspecific hybrids in Agropyron. II (in Jap.). Jap. Jour. Genet. 18: 133-135.

Matsumura, S. 1948. Genomanalyse bei Agropyrum, als verwandtschaftlicher Gattung von Triticum. I. Artbastarde bei Agropyrum. (in Jap.). Oguma Commemoration Volume on Cytology and Genetics: 116-124.

Matsumura, S., S. Sakamoto and T. Tateoka. 1956. Agropyron and its related genera. In Land and Crops of Nepal Himalaya. Scientific Results of the Japanese Expedition to Nepal Himalaya, 1952-1953, Vol. II: 249-253.

McClintock, B. 1929. A method for making aceto-carmine smear permanent. Stain Tech. 4: 53-56.

Miquel, F. A. C. 1866-1867. Prolusio Florae Japanicae. pp. 392.

Moriya, A. and A. Kondo 1950. Cytological studies of forage plants, I. Grasses. Jap. Jour. Genet. 25: 126-131.

Nakajima, G. 1936. Chromosome numbers in some crops and wild angiosperms. Jap. Jour. Genet. 12: 211-218.

Nakajima, K. 1952. An enumeration of plants hitherto known from Fukuoka Prefecture. (Mimeographed).

Ohwi, J. 1937a. Plantae novae Japonicae. IV. Jour. Jap. Bot. 13: 332-343.

Ohwi, J. 1937b. Sertum Japonicum (in Jap.). Acta Phytotax. et Geobot. 6: 53-56.

Ohwi, J. 1941. Gramina Japonica I. (in Jap.) Acta Phytotax. et Geobot. 10: 94-135.

Ohwi, J. 1942a. Gramina Japonica. IV. Acta Phytotax. et Geobot. 11: 145-193.

Ohwi, J. 1942b. Symbolae ad Floram Asiae Orientalis. 18. Acta Phytotax. et Geobot. 11: 249-265

Ohwi, J. 1953. Flora of Japan (in Jap.). Shibundo, Tokyo, pp. 1383.

Ohwi, J. and S. Sakamoto 1964. Taxonomy, ecology and distribution of Agropyron humidum Ohwi et Sakamoto. Jour. Jap. Bot. 39: 109-114.

Ono, H. and T. Tateoka 1953. Karyotaxonomy in Poaceae. I. Chromosomes and taxonomic relations in some Japanese grasses. Bot. Mag. Tokyo 66: 18-27.

Östergren, G. 1940a. Cytology of Agropyron junceum, A. repens and their spontaneous hybrids. Hereditas 26: 305-316.

Östergren, G. 1940. On the morphology of Agropyron junceum (L.) P. B., A. repens (L.) P. B. and their spontaneous hybrids. Botaniska Notiser 1940: 133-143.

Sakamoto, S. 1957. Ecogenetical studies in Agropyron. Ann. Rep. Nat. Inst. Genet., Japan No. 8: 34-35.

Sakamoto, S. 1958. An ecotype of Agropyron tsukushiense var. transiens and natural hybrids between A. tsukushiense var. transiens and A. Mayebaranum var. Mayebaranum (in Jap.). In Abst. of Papers Presented at the 23rd Ann. Meet. Bot. Soc. Japan: 96.

Sakamoto, S. 1961. An early ecotype of Agropyron tsukushiense var. transiens Ohwi (in Jap.). Seiken Zihô 12: 45-58.

Sakamoto, S. 1964a. Cytogenetic studies in the tribe Triticeae. I. A polyhaploid plant of Agropyron tsukushiense var. transiens found in a state of nature. Jap. Jour. Genet. 39: (in press).

Sakamoto, S. 1964b. Cytogenetic problems in Agropyron hybrids. Seiken Zihô 16: (in press).

Sax, K. 1935. The cytological analysis of species hybrids. Bot. Rev. 1: 100-117.

Simonet, M. 1934. Sur la valeur taxonomique de l'Agropyrum acutum Roehm. et Schult. Contrôle cytologique. Bull. Soc. Bot. France 81: 801-814.

Simonet, M. 1935. Contributions à l'étude cytologique et génétique de quelques Agropyrum. Comp. Rend. Acad. Sci. 201: 1210-1212.

Stebbins, G. L. 1945. The cytological analysis of species hybrids. II. Bot. Rev. 11: 463-486.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York, pp. 643.

Stebbins, G. L., Jr., and P. T. Pun 1953. Artificial and natural hybrids in the Gramineae, tribe Hordeae. V. Diploid hybrids of Agropyron. Amer. Jour Bot. 40: 444-449.

Stebbins, G. L., Jr., and R. Singh 1950. Artificial and natural hybrids in the Gramineae, tribe Hordeae. IV. Two triploid hybrids of Agropyron and Elymus. Amer. Jour. Bot 37: 388-393

Stebbins, G. L. and L. A. Snyder 1956 Artificial and natural hybrids in the Gramineae, tribe Hordeae, IX. Hybrids between western and eastern North American species. Amer. Jour. Bot 43: 305-312

Stebbins, G. L., Jr., and A. Vaarama 1954. Artificial and natural hybrids in the Gramineae, tribe Hordeae VII. Hybrids and allopolyploids between Elymus glaucus and Sitanion ssp. Genetics 39: 378-395

Stebbins, G. L., Jr., J. I. Valencia and R. M. Valencia 1946. Artificial and natural hybrids in the Gramineae, tribe Hordeae I. Elymus, Sitanion and Agropyron. Amer. Jour. Bot. 33: 338-351.

Tateoka, T. 1954. Karyotaxonomy in Poaceae II Somatic chromosomes of some species. Cytologia 19: 317-328.

Tateoka, T. 1956 On morphological convergence between Brachypodium sylvaticum and Agropyron yezoense. Cytologia 21: 146-152

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